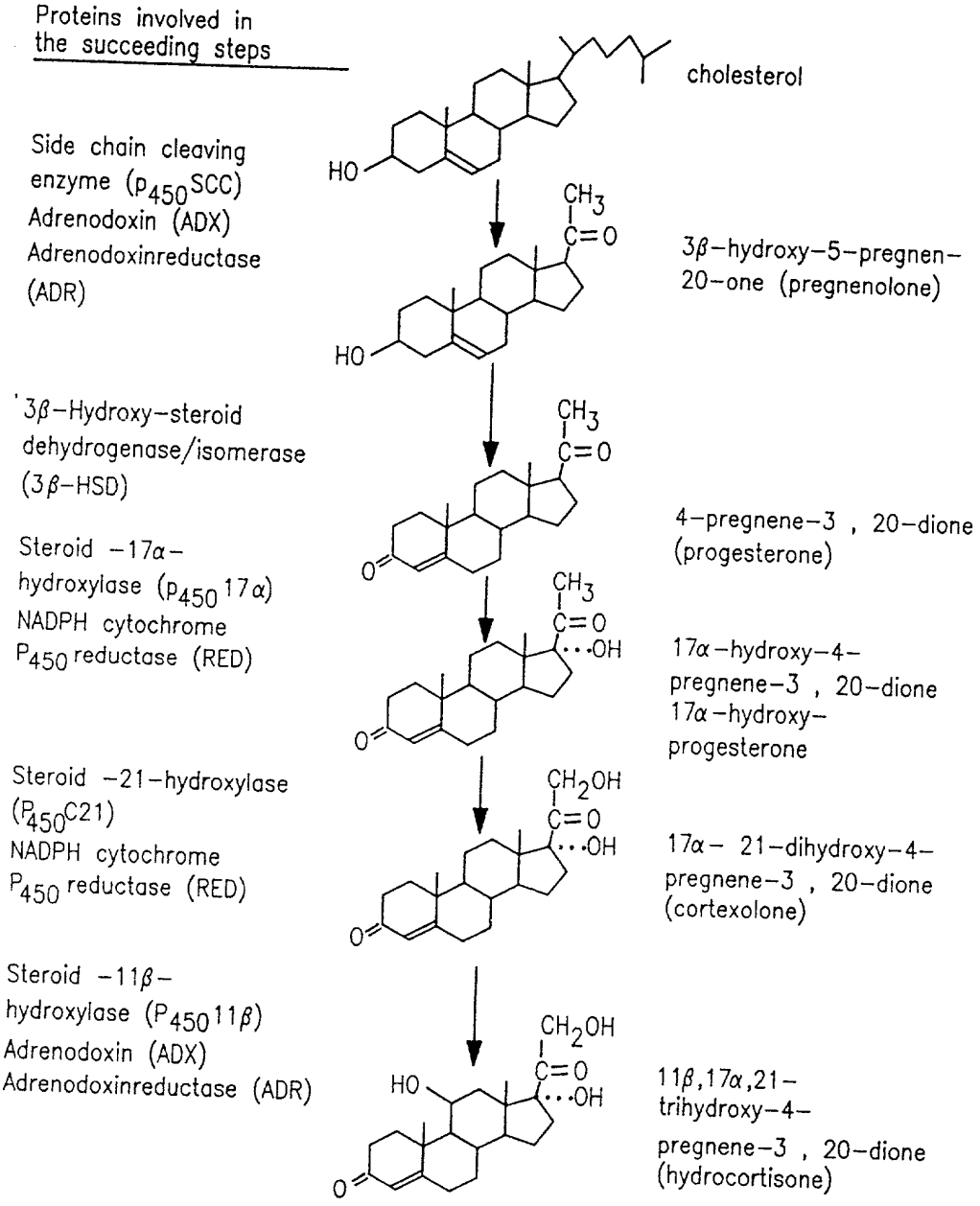

Proteins involved in the succeeding steps
cholesterol
Side chain cleaving enzyme (P_{450}SCC)
Adrenodoxin (ADX)
Adrenodoxinreductase (ADR)
3β-hydroxy-5-pregnen-20-one (pregnenolone)
3β-Hydroxy-steroid dehydrogenase/isomerase (3β-HSD)
4-pregnene-3 , 20-dione (progesterone)
Steroid -17α-hydroxylase (P_{450} 17α)
NADPH cytochrome P_{450} reductase (RED)
17α-hydroxy-4-pregnene-3 , 20-dione
17α-hydroxy-progesterone
Steroid -21-hydroxylase (P_{450}C21)
NADPH cytochrome P_{450} reductase (RED)
17α- 21-dihydroxy-4-pregnene-3 , 20-dione (cortexolone)
Steroid -11β-hydroxylase (P_{450}11β)
Adrenodoxin (ADX)
Adrenodoxinreductase (ADR)
11β,17α,21-trihydroxy-4-pregnene-3 , 20-dione (hydrocortisone)

FIG. 1

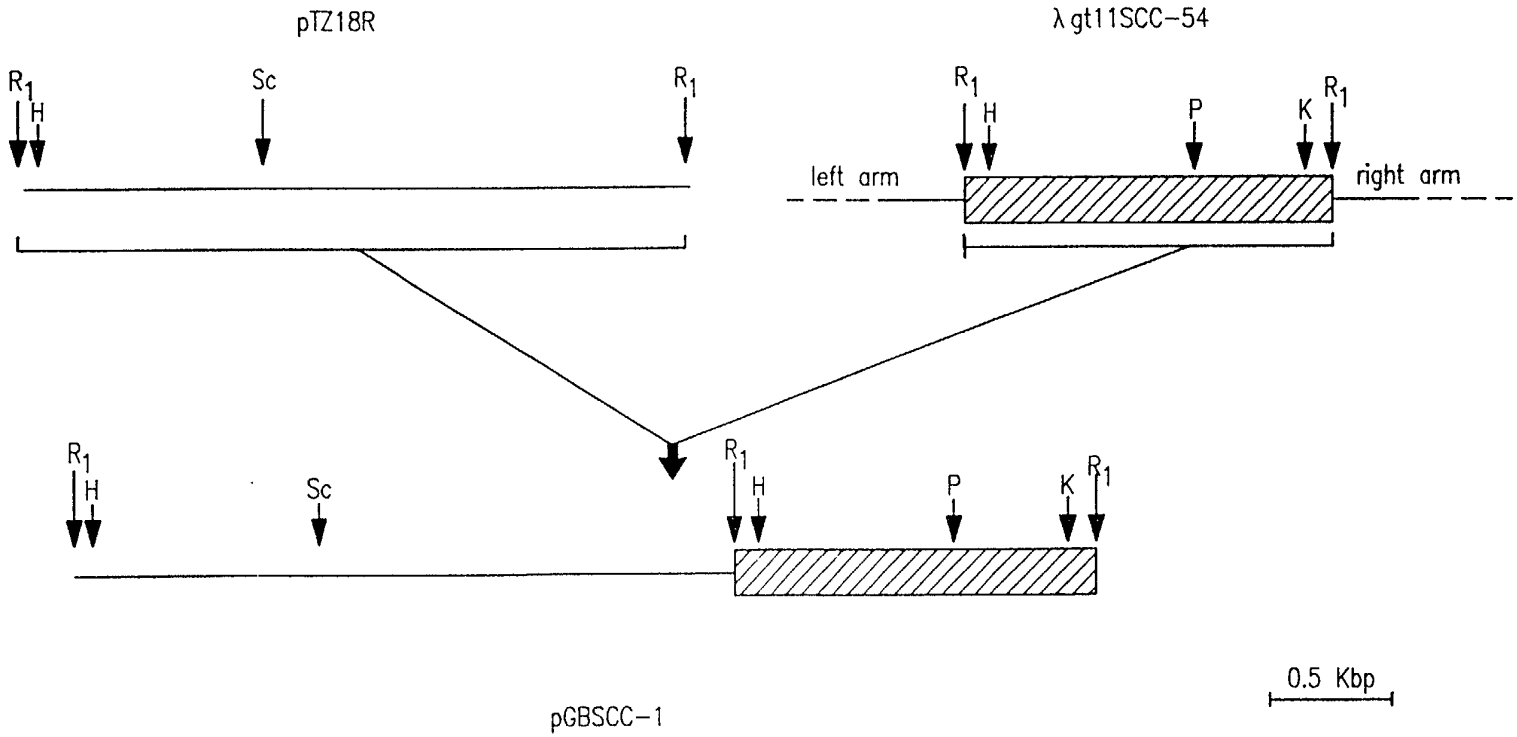

pTZ18R
λgt11SCC-54
R1
H
Sc
R1
R1
H
P
K
R1
left arm
right arm
R1
H
Sc
R1
H
P
K
R1
0.5 Kbp
pGBSCC-1

FIG. 2

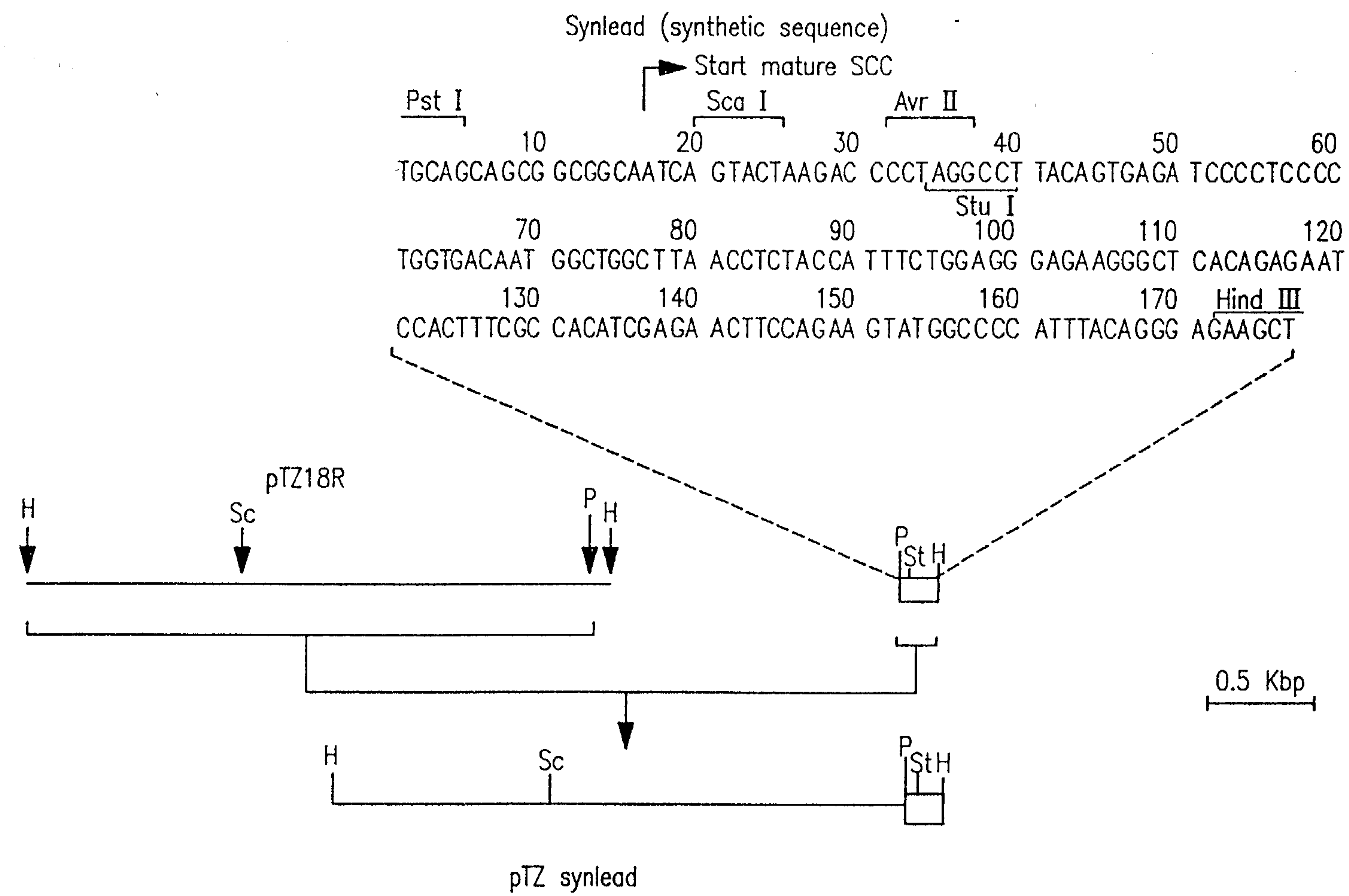

Synlead (synthetic sequence)
Start mature SCC
Pst I
Sca I
Avr II
10 20 30 40 50 60
TGCAGCAGCG GCGGCAATCA GTACTAAGAC CCCTAGGCCT TACAGTGAGA TCCCCTCCCC
Stu I
70 80 90 100 110 120
TGGTGACAAT GGCTGGCTTA ACCTCTACCA TTTCTGGAGG GAGAAGGGCT CACAGAGAAT
130 140 150 160 170
CCACTTTCGC CACATCGAGA ACTTCCAGAA GTATGGCCCC ATTTACAGGG AGAAGCT
Hind III
pTZ18R
H
Sc
P
H
P
St
H
0.5 Kbp
H
Sc
P
St
H
pTZ synlead

FIG. 3

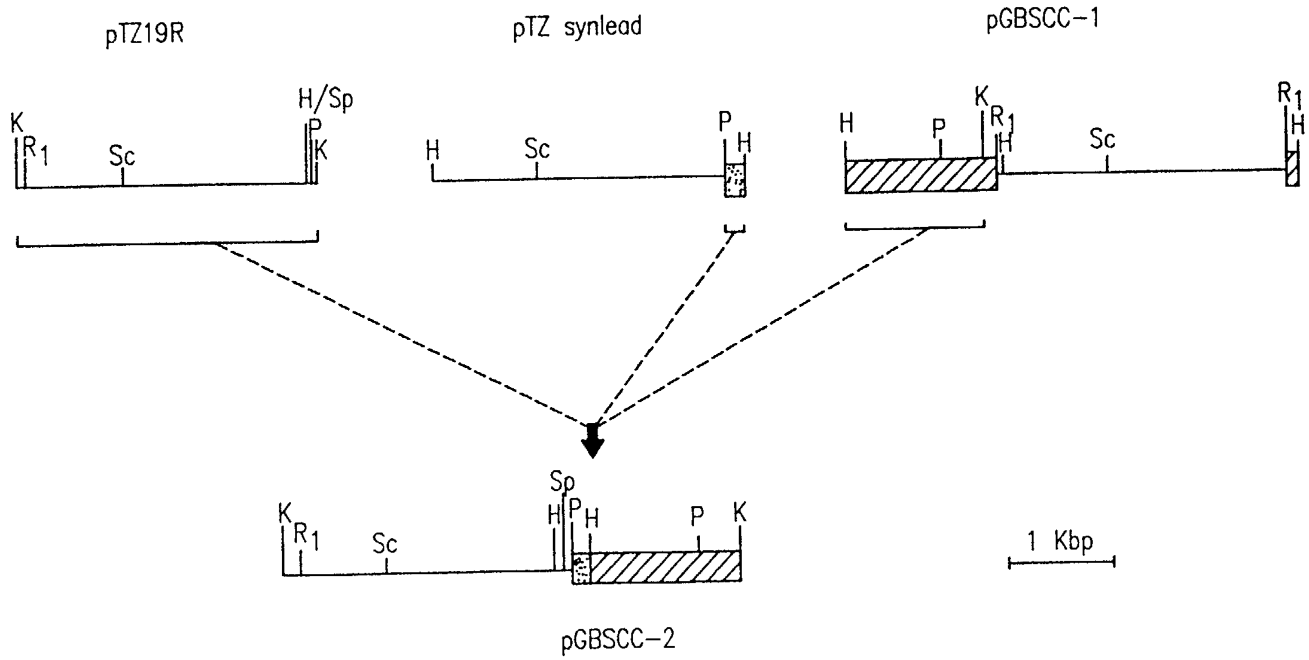

pTZ19R
pTZ synlead
pGBSCC-1
K
R1
Sc
H/Sp
P
K
H
Sc
P
H
H
P
K
R1
H
Sc
R1
H
K
R1
Sc
Sp
H
P
H
P
K
pGBSCC-2
1 Kbp

FIG. 4

```
                              30                            60                            90
AATTCACCTC GAAAGGAAGC TGATAAACCG ATACAATTAA AGGCTCCTTT TGGAGCCTTT TTTTTTGGAG ATTTTCAACG TGAAAAAATT

                             120                           150                           180
ATTATTCGCA ATTCCAAGCT AATTCACCTC GAAAGCAAGC TGATAAACCG ATACAATTAA AGGCTCCTTT TGGAGCCTTT TTTTTTGGAG

                             210                           240                           270
ATTTTCAACG TGAAAAAATT ATTATTCGCA ATTCCAAGCT CTGCCTCGCG CGTTTCGGTG ATGACGGTGA AAACCTCTGA CACATGCAGC

                             300                           330                           360
TCCCGGAGAC GGTCACAGCT TGTCTGTAAG CGGATGCAGA TCACGCGCCC TGTAGCGGCG CATTAAGCGC GGCGGGTGTG GTGGTTACGC

                             390                           420                           450
GCAGCGTGAC CGCTACACTT GCCAGCGCCC TAGCGCCCGC TCCTTTCGCT TTCTTCCCTT CCTTTCTCGC CACGTTCGCC GGCTTTCCCC

                             480                           510                           540
GTCAAGCTCT AAATCGGGGG CTCCCTTTAG GGTTCCGATT TAGTGCTTTA CGGCACCTCG ACCCCAAAAA ACTTGATTAG GGTGATGGTT

                             570                           600                           630
CACGTAGTGG GCCATCGCCC TGATAGACGG TTTTTCGCCC TTTGACGTTG GAGTCCACGT TCTTTAATAG TGGACTCTTG TTCCAAACTG

                             660                           690                           720
GAACAACACT CAACCCTATC TCGGTCTATT CTTTTGATTT ATAAGGGATT TTGCCGATTT CGGCCTATTG GTTAAAAAAT GAGCTGATTT

                             750                           780                           810
AACAAAAATT TAACGCGAAT TTTAACAAAA TATTAACGTT TACAATTTGA TCTGCGCTCG GTCGTTCGGC TGCGGCGAGC GGTATCAGCT

                             840                           870                           900
CACTCAAAGG CGGTAATACG GTTATCCACA GAATCAGGGG ATAACGCAGG AAAGAACATG TGAGCAAAAG GCCAGCAAAA GGCCAGGAAC

                             930                           960                           990
CGTAAAAAGG CCGCGTTGCT GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAC GCTCAAGTCA GAGGTGGCGA

                            1020                          1050                          1080
AACCCGACAG GACTATAAAG ATACCAGGCG TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC
```

FIG. 5A

```
                               1110                                  1140                                  1170
CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG

                               1200                                  1230                                  1260
CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC

                               1290                                  1320                                  1350
TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT GCTACAGAGT TCTTGAAGTG GTGGCCTAAC

                               1380                                  1410                                  1440
TACGGCTACA CTAGAAGGAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTTC GGAAAAAGAG TTGGTAGCTC TTGATCCGGC

                               1470                                  1500                                  1530
AAACAAACCA CCGCTGGTAG CGGTGGTTTT TTTGTTTGCA AGCAGCAGAT TACGCGCAGA AAAAAAGGAT CTCAAGAAGA TCCTTTGATC

                               1560                                  1590                                  1620
TTTTCTACGG GGTCTGACGC TCAGTGGAAC GAAAACTCAC GTTAAGGGAT TTTGGTCATG AGATTATCAA AAAGGATCTT CACCTAGATC

                               1650                                  1680                                  1710
CTTTTAAATT AAAAATGAAG TTTTAAATCA ATCTAAAGTA TATATGAGTA AACTTGGTCT GACAGTTACC AATGCTTAAT CAGTGAGGCA

                               1740                                  1770                                  1800
CCTATCTCAG CGATCTGTCT ATTTCGTTCA TCCATAGTTG CCTGACTCCC CGTCGTGTAG ATAACTACGA TACGGGAGGG CTTACCATCT

                               1830                                  1860                                  1890
GGCCCCAGTG CTGCAATGAT ACCGCGAGAC CCACGCTCAC CGGCTCCAGA TTTATCAGGA ATAAACCAGC CAGCCGGAAG GGCCGAGCGC

                               1920                                  1950                                  1980
AGAAGTGGTC CTGCAACTTT ATCCGCCTCC ATCCAGTCTA TTAATTGTTG CCGGGAAGCT AGAGTAAGTA GTTCGCCAGT TAATAGTTTG

                               2010                                  2040                                  2070
CGCAACGTTG TTGCCATTGC TGCAGGCATC GTGGTGTCAC GCTCGTCGTT TGGTATGGCT TCATTCAGCT CCGGTTCCCA ACGATCAAGG
```

FIG. 5B

```
                                2100                                              2130                                              2160
CGAGTTACAT GATCCCCCAT GTTGTGCAAA AAAGCGGTTA GCTCCTTCGG TCCTCCGATC GTTGTCAGAA GTAAGTTGGC CGCAGTGTTA
                                2190                                              2220                                              2250
TCACTCATGG TTATGGCAGC ACTGCATAAT TCTCTTACTG TCATGCCATC CGTAAGATGC TTTTCTGTGA CTGGTGAGTA CTCAACCAAG
                                2280                                              2310                                              2340
TCATTCTGAG AATAGTGTAT GCGGCGACCG AGTTGCTCTT GCCCGGCGTC AACACGGGAT AATACCGCGC CACATAGCAG AACTTTAAAA
                                2370                                              2400                                              2340
GTGCTCATCA TTGGAAAACG TTCTTCGGGG CGAAAACTCT CAAGGATCTT ACCGCTGTTG AGATCCAGTT CGATGTAACC CACTCGTGCA
                                2460                                              2490                                              2520
CCCAACTGAT CTTCAGCATC TTTTACTTTC ACCAGCGTTT CTGGGTGAGC AAAAACAGGA AGGCAAAATG CCGCAAAAAA GGGAATAAGG
                                2550                                              2580                                              2610
GCGACACGGA AATGTTGAAT ACTCATACTC TTCCTTTTTC AATATTATTG AAGCAGACAG TTTTATTGTT CATGATGATA TATTTTTATC
                                2640                                              2670                                              2700
TTGTGCAATG TAACATCAGA GATTTTGAGA CACAACGTGG CTTTGTTGAA TAAATCGAAC TTTTGCTGAG TTGACTCCCC GCGCGCGATG
                                2730                                              2760                                              2790
GGTCGAATTT GCTTTCGAAA AAAAAGCCCG CTCATTAGGC GGGCTAAAAA AAAGCCCGCT CATTAGGCGG GCTCGAATTT CTGCCATTCA
                                2820                                              2850                                              2880
TCCGCTTATT ATCACTTATT CAGGCGTAGC AACCAGGCGT TTAAGGGCAC CAATAACTGC CTTAAAAAAA TTACGCCCCG CCCTGCCACT
                                2910                                              2940                                              2970
CATCGCAGTA CTGTTGTAAT TCATTAAGCA TTCTGCCGAC ATGGAAGCCA TCACAGACGG CATGATGAAC CTGAATCGCC AGCGGCATCA
```

FIG. 5C

```
                                 3000                                    3030                                    3060
GCACCTTGTC GCCTTGCGTA TAATATTTGC CCATAGTGAA AACGGGGGCG AAGAAGTTGT CCATATTCGC CACGTTTAAA TCAAAACTGG
                                 3090                                    3120                                    3150
TGAAACTCAC CCAGGGATTG GCTGAGACGA AAAACATATT CTCAATAAAC CCTTTAGGGA AATAGGCCAG GTTTTCACCG TAACACGCCA
                                 3180                                    3210                                    3240
CATCTTGCGA ATATATGTGT AGAAACTGCC GGAAATCGTC GTGGTATTCA CTCCAGAGCG ATGAAAACGT TTCAGTTTGC TCATGGAAAA
                                 3270                                    3300                                    3330
CGGTGTAACA AGGGTGAACA CTATCCCATA TCACCAGCTC ACCGTCTTTC ATTGCCATAC GAAATTCCGG ATGAGCATTC ATCAGGCGGG
                                 3360                                    3390                                    3420
CAAGAATGTG AATAAAGGCC GGATAAAACT TGTGCTTATT TTTCTTTACG GTCTTTAAAA AGGCCGTAAT ATCCAGCTAA ACGGTCTGGT
                                 3450                                    3480                                    3510
TATAGGTACA TTGAGCAACT GACTGAAATG CCTCAAAATG TTCTTTACGA TGCCATTGGG ATATATCAAC GGTGGTATAT CCAGTGATTT
                                 3540                                    3570                                    3600
TTTTCTCCAT TTTAGCTTCC TTAGCTCCTG AAAATCTCGA TAACTCAAAA AATACGCCCG GTAGTGATCT TATTTCATTA TGGTGAAAGT
                                 3630                                    3660                                    3690
TGGAACCTCT TACGTGCCGA TCAACGTCTC ATTTTCGCCA AAAGTTGGCC CAGGGCTTCC CGGTATCAAC AGGGACACCA GGATTTATTT
                                 3720                                    3750                                    3780
ATTCTGCGAA GTGATCTTCC GTCACAGGTA TTTATTCGAA GACGAAAGGG CATCGCGCGC GGGGAATTCC CGGGAGAGCT CGATATCGCA
                                 3810                                    3840                                    3870
TGCGGTACCT CTAGAAGAAG CTTGGAGACA AGGTAAAGGA TAAAACAGCA CAATTCCAAG AAAAACACGA TTTAGAACCT AAAAAGAACG
                                 3900                                    3930                                    3960
AATTTGAACT AACTCATAAC CGAGAGGTAA AAAAAGAACG AAGTCGAGAT CAGGGAATGA GTTTATAAAA TAAAAAAAGC ACCTGAAAAG
```

FIG. 5D

```
                              3990                           4020                           4050
GTGTCTTTTT TTGATGGTTT TGAACTTGTT CTTTCTTATC TTGATACATA TAGAAATAAC GTCATTTTTA TTTTAGTTGC TGAAAGGTGC
                              4080                           4110                           4140
GTTGAAGTGT TGGTATGTAT GTGTTTTAAA GTATTGAAAA CCCTTAAAAT TGGTTGCACA GAAAAACCCC ATCTGTTAAA GTTATAAGTG
                              4170                           4200                           4230
ACTAAACAAA TAACTAAATA GATGGGGGTT TCTTTTAATA TTATGTGTCC TAATAGTAGC ATTTATTCAG ATGAAAAATC AAGGGTTTTA
                              4260                           4290                           4320
GTGGACAAGA CAAAAAGTGG AAAAGTGAGA CCATGGAGAG AAAAGAAAAT CGCTAATGTT GATTACTTTG AACTTCTGCA TATTCTTGAA
                              4350                           4380                           4410
TTTAAAAAGG CTGAAAGAGT AAAAGATTGT GCTGAAATAT TAGAGTATAA ACAAAATCGT GAAACAGGCG AAAGAAAGTT GTATCGAGTG
                              4440                           4470                           4500
TGGTTTTGTA AATCCAGGCT TTGTCCAATG TGCAACTGGA GGAGAGCAAT GAAACATGGC ATTCAGTCAC AAAAGGTTGT TGCTGAAGTT
                              4530                           4560                           4590
ATTAAACAAA AGCCAACAGT TCGTTGGTTG TTTCTCACAT TAACAGTTAA AAATGTTTAT GATGGCGAAG AATTAAATAA GAGTTTGTCA
                              4620                           4650                           4680
GATATGGCTC AAGGATTTCG CCGAATGATG CAATATAAAA AAATTAATAA AAATCTTGTT GGTTTTATGC GTGCAACGGA AGTGACAATA
                              4710                           4740                           4770
AATAATAAAG ATAATTCTTA TAATCAGCAC ATGCATGTAT TGGTATGTGT GGAACCAACT TATTTTAAGA ATACAGAAAA CTACGTGAAT
                              4800                           4830                           4860
CAAAAACAAT GGATTCAATT TTGGAAAAAG GCAATGAAAT TAGACTATGA TCCAAATGTA AAAGTTCAAA TGATTCGACC GAAAAATAAA
                              4890                           4920                           4950
TATAAATCGG ATATACAATC GGCAATTGAC GAAACTGCAA AATATCCTGT AAAGGATACG GATTTTATGA CCGATGATGA AGAAAAGAAT
                              4980                           5010                           5040
TTGAAACGTT TGTCTGATTT GGAGGAAGGT TTACACCGTA AAAGGTTAAT CTCCTATGGT GGTTTGTTAA AAGAAATACA TAAAAAATTA
```

FIG. 5E

```
                                5070                                     5100                                     5130
AACCTTGATG ACACAGAAGA AGGCGATTTG ATTCATACAG ATGATGACGA AAAAGCCGAT GAAGATGGAT TTTCTATTAT TGCAATGTGG

                                5160                                     5190                                     5220
AATTGGGAAC GGAAAAATTA TTTTATTAAA GAGTAGTTCA ACAAACGGGC CAGTTTGTTG AAGATTAGAT GCTATAATTG TTATTAAAAG

                                5250                                     5280                                     5310
GATTGAAGGA TGCTTAGGAA GACGAGTTAT TAATAGCTGA ATAAGAACGG TGCTCTCCAA ATATTCTTAT TTAGAAAAGC AAATCTAAAA

                                5340                                     5370                                     5400
TTATCTGAAA AGGGAATGAG AATAGTGAAT GGACCAATAA TAATGACTAG AGAAGAAAGA ATGAAGATTG TTCATGAAAT TAAGGAACGA

                                5430                                     5460                                     5490
ATATTGGATA AATATGGGGA TGATGTTAAG GCTATTGGTG TTTATGGCTC TCTTGGTCGT CAGACTGATG GGCCCTATTC GGATATTGAG

                                5520                                     5550                                     5580
ATGATGTGTG TCATGTCAAC AGAGGAAGCA GAGTTCAGCC ATGAATGGAC AACCGGTGAG TGGAAGGTGG AAGTGAATTT TGATAGCGAA

                                5610                                     5640                                     5670
GAGATTCTAC TAGATTATGC ATCTCAGGTG GAATCAGATT GGCCGCTTAC ACATGGTCAA TTTTTCTCTA TTTTGCCGAT TTATGATTCA

                                5700                                     5730                                     5760
GGTGGATACT TAGAGAAAGT GTATCAAACT GCTAAATCGG TAGAAGCCCA AACGTTCCAC GATGCGATTT GTGCCCTTAT CGTAGAAGAG

                                5790                                     5820                                     5850
CTGTTTGAAT ATGCAGGCAA ATGGCGTAAT ATTCGTGTGC AAGGACCGAC AACATTTCTA CCATCCTTGA CTGTACAGGT AGCAATGGCA

                                5880                                     5910                                     5940
GGTGCCATGT TGATTGGTCT GCATCATCGC ATCTGTTATA CGACGAGCGC TTCGGTCTTA ACTGAAGCAG TTAAGCAATC AGATCTTCCT

                                5970                                     6000                                     6030
TCAGGTTATG ACCATCTGTG CCAGTTCGTA ATGTCTGGTC AACTTTCCGA CTCTGAGAAA CTTCTGGAAT CGCTAGAGAA TTTCTGGAAT

                                6060                                     6090                                     6120
GGGATTCAGG AGTGGACAGA ACGACACGGA TATATAGTGG ATGTGTCAAA ACGCATACCA TTTTGAACGA TGACCTCTAA TAATTGTTAA
```

FIG. 5F

```
                                                   6150                                                   6180                                                   6210
TCATGTTGGT TACGTATTTA TTAACTTCTC CTAGTATTAG TAATTATCAT GGCTGTCATG GCGCATTAAC GGAATAAAGG GTGTGCTTAA

                                                   6240                                                   6270                                                   6300
ATCGGGCCAT TTTGCGTAAT AAGAAAAAGG ATTAATTATG AGCGAATTGA ATTAATAATA AGGTAATAGA TTTACATTAG AAAATGAAAG

                                                   6330                                                   6360                                                   6390
GGGATTTTAT GCGTGAGAAT GTTACAGTCT ATCCCGGCAT TGCCAGTCGG GGATATTAAA AAGAGTATAG GTTTTTATTG CGATAAACTA

                                                   6420                                                   6450                                                   6480
GGTTTCACTT TGGTTCACCA TGAAGATGGA TTCGCAGTTC TAATGTGTAA TGAGGTTCGG ATTCATCTAT GGGAGGCAAG TGATGAAGGC

                                                   6510                                                   6540                                                   6570
TGGCGCTCTC GTAGTAATGA TTCACCGGTT TGTACAGGTG CGGAGTCGTT TATTGCTGGT ACTGCTAGTT GCCGCATTGA AGTAGAGGGA

                                                   6600                                                   6630                                                   6660
ATTGATGAAT TATATCAACA TATTAAGCCT TTGGGCATTT TGCACCCCAA TACATCATTA AAAGATCAGT GGTGGGATGA ACGAGACTTT

                                                   6690                                                   6720                                                   6750
GCAGTAATTG ATCCCGACAA CAATTTGATT AGCTTTTTTC AACAAATAAA AAGCTAAAAT CTATTATTAA TCTGTTCAGC AATCGGGCGC

                                                   6780                                                   6810                                                   6840
GATTGCTGAA TAAAAGATAC GAGAGACCTC TCTTGTATCT TTTTTATTTT GAGTGGTTTT GTCCGTTACA CTAGAAAACC GAAAGACAAT

                                                   6870                                                   6900                                                   6930
AAAAATTTTA TTCTTGCTGA GTCTGGCTTT CGGTAAGCTA GACAAAACGG ACAAAATAAA AATTGGCAAG GGTTTAAAGG TGGAGATTTT

                                                   6960                                                   6990                                                   7020
TTGAGTGATC TTCTCAAAAA ATACTACCTG TCCCTTGCTG ATTTTTAAAC GAGCACGAGA GCAAAACCCC CCTTTGCTGA GGTGGCAGAG

                                                   7050                                                   7080                                                   7110
GGCAGGTTTT TTTGTTTCTT TTTTCTCGTA AAAAAAAGAA AGGTCTTAAA GGTTTTATGG TTTTGGTCGG CACTGCCGAC AGCCTCGCAG

                                                   7140                                                   7170                                                   7200
GACACACACT TTATGAATAT AAAGTATAGT GTGTTATACT TTACTTGGAA GTGGTTGCCG GAAAGAGCGA AAATGCCTCA CATTTGTGCC

                                                   7230                                                   7260                                                   7290
ACCTAAAAAG GAGCGATTTA CATATGAGTT ATGCAGTTTG TAGAATGCAA AAAGTGAAAT CAGGGGGATC CTCTAGAGTC GAGCTCAAGC

                                                   7320
TAGCTTGGTA CGTACCAGAT CTGAGATCAC GCGTTCTAGA GGTCGA
```

FIG. 5G

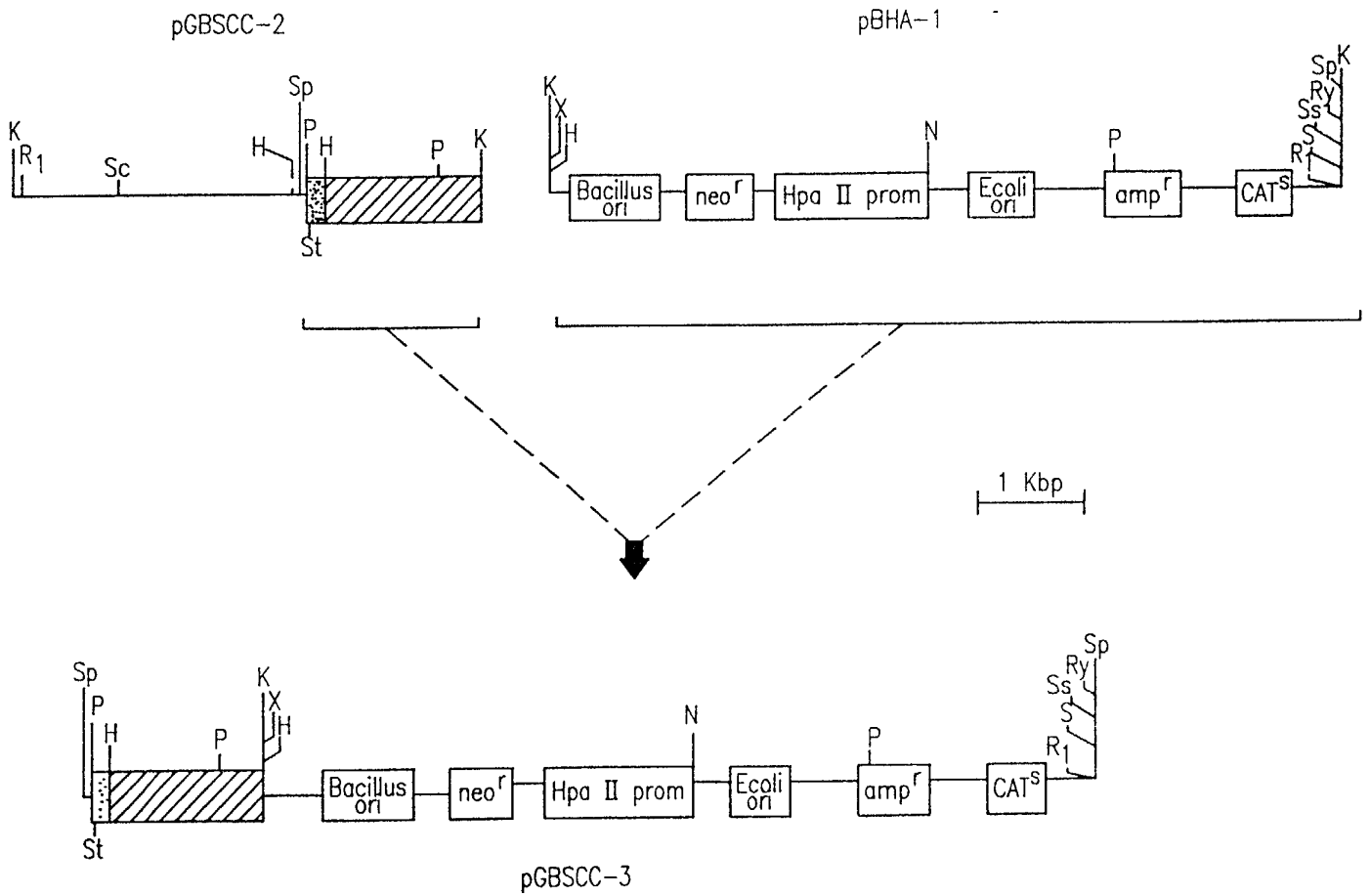

pGBSCC-2
pBHA-1
K
R1
Sc
H
Sp
P
H
P
K
St
K
X
H
N
P
Sp
K
Ry
Ss
S
R
Bacillus ori
neo^r
Hpa II prom
Ecoli ori
amp^r
CAT^s
1 Kbp
Sp
P
H
P
K
X
H
St
N
P
Sp
Ry
Ss
S
R1
Bacillus ori
neo^r
Hpa II prom
Ecoli ori
amp^r
CAT^s
pGBSCC-3

FIG. 6

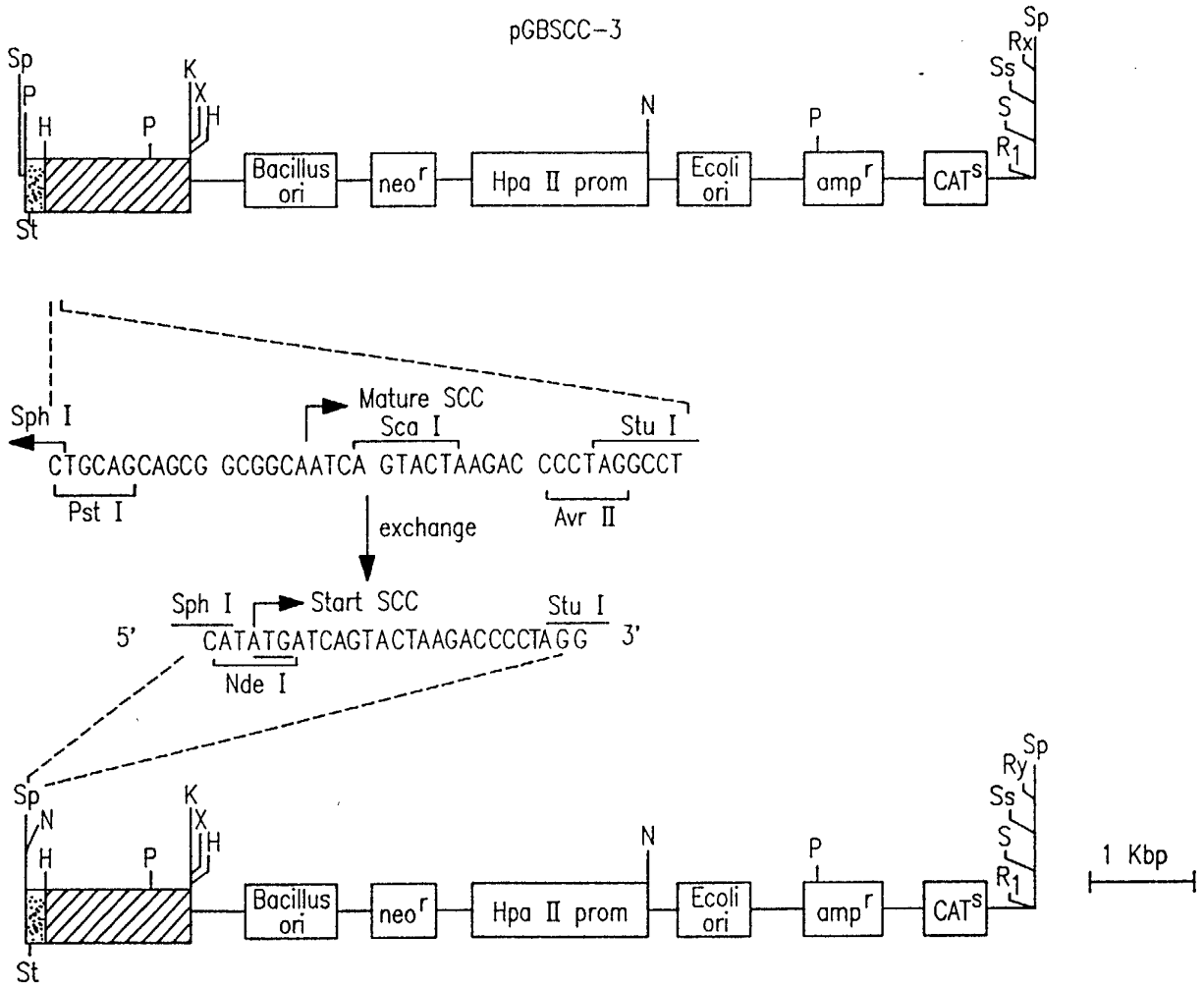

pGBSCC-3
Sp
P
H
K
X
H
P
St
Bacillus ori
neo^r
N
Hpa II prom
Ecoli ori
P
amp^r
CAT^s
Sp
Rx
Ss
S
R1
Sph I
Mature SCC
Sca I
Stu I
CTGCAGCAGCG GCGGCAATCA GTACTAAGAC CCCTAGGCCT
Pst I
exchange
Avr II
Sph I
Start SCC
Stu I
5'
CATATGATCAGTACTAAGACCCCTAGG
3'
Nde I
Sp
N
H
K
X
H
P
St
Bacillus ori
neo^r
N
Hpa II prom
Ecoli ori
P
amp^r
CAT^s
Sp
Ry
Ss
S
R1
1 Kbp
pGBSCC-4

FIG. 7

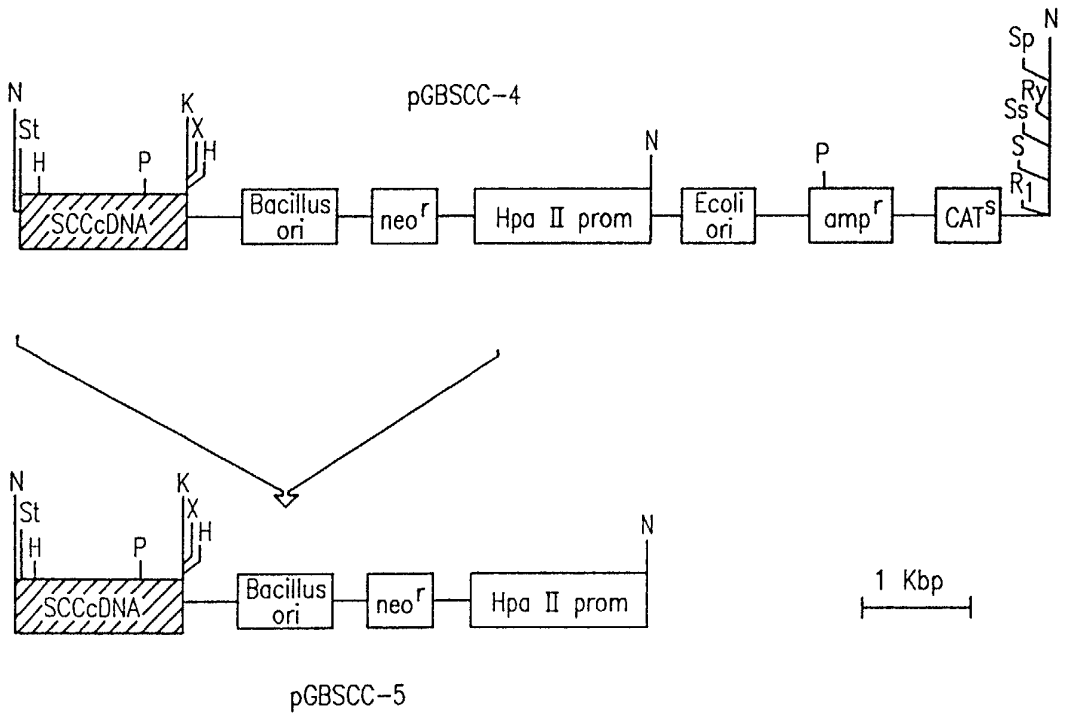

pGBSCC-4
N
St
H
P
K
X
H
SCCcDNA
Bacillus ori
neo^r
N
Hpa II prom
Ecoli ori
P
amp^r
CAT^s
Sp
N
Ry
Ss
S
R
1
pGBSCC-5
N
St
H
P
K
X
H
SCCcDNA
Bacillus ori
neo^r
N
Hpa II prom
1 Kbp

FIG. 8

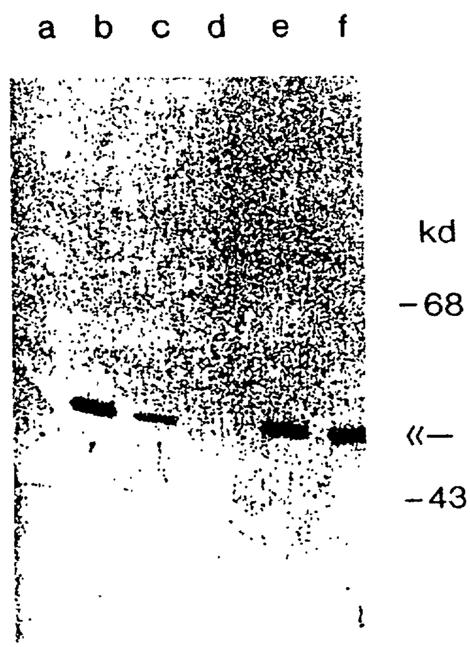


FIG. 9

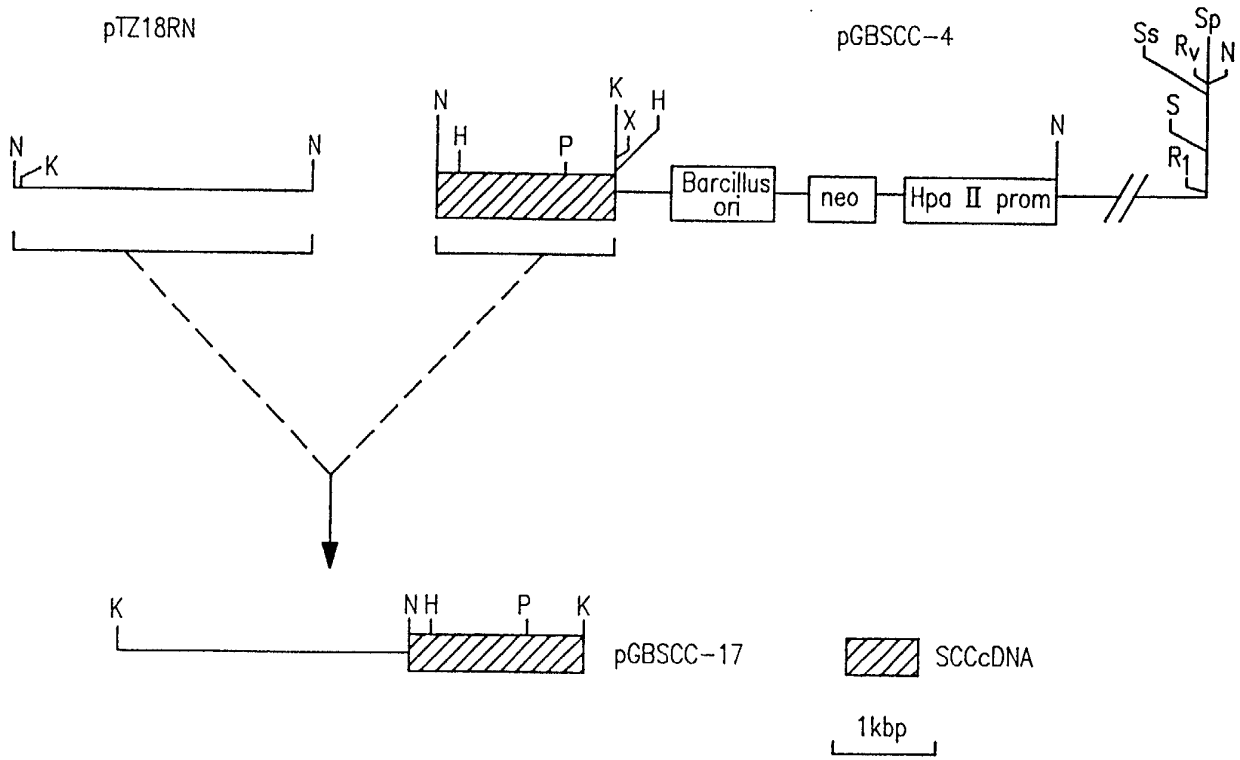

pTZ18RN
N
K
N
pGBSCC-4
N
H
P
K
X
H
Barcillus ori
neo
Hpa II prom
N
Ss
Rv
Sp
N
S
R1
K
NH
P
K
pGBSCC-17
SCCcDNA
1kbp

FIG. 10

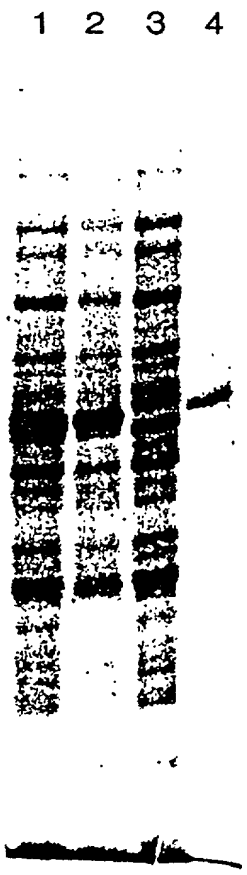


FIG. IIA

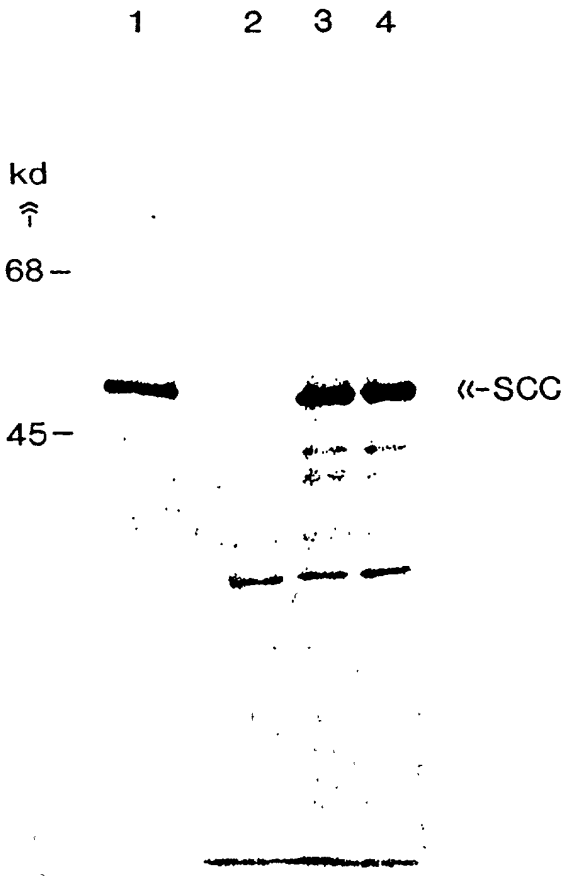
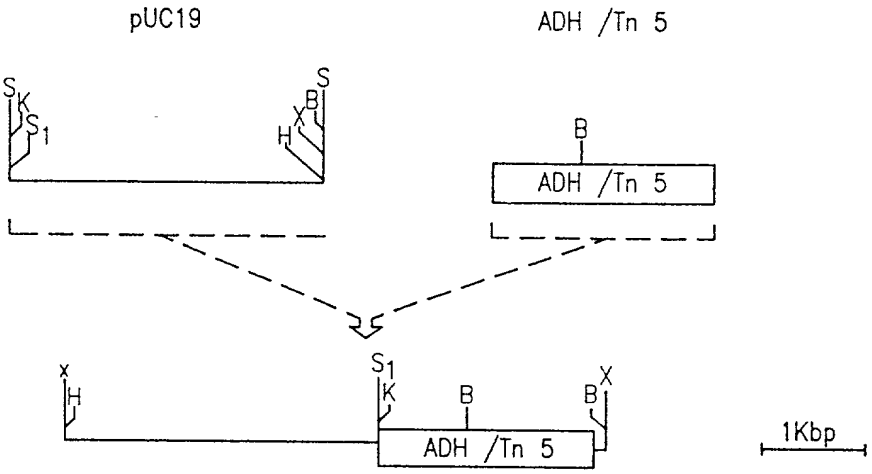


FIG. IIB



pUCG418

FIG. 12

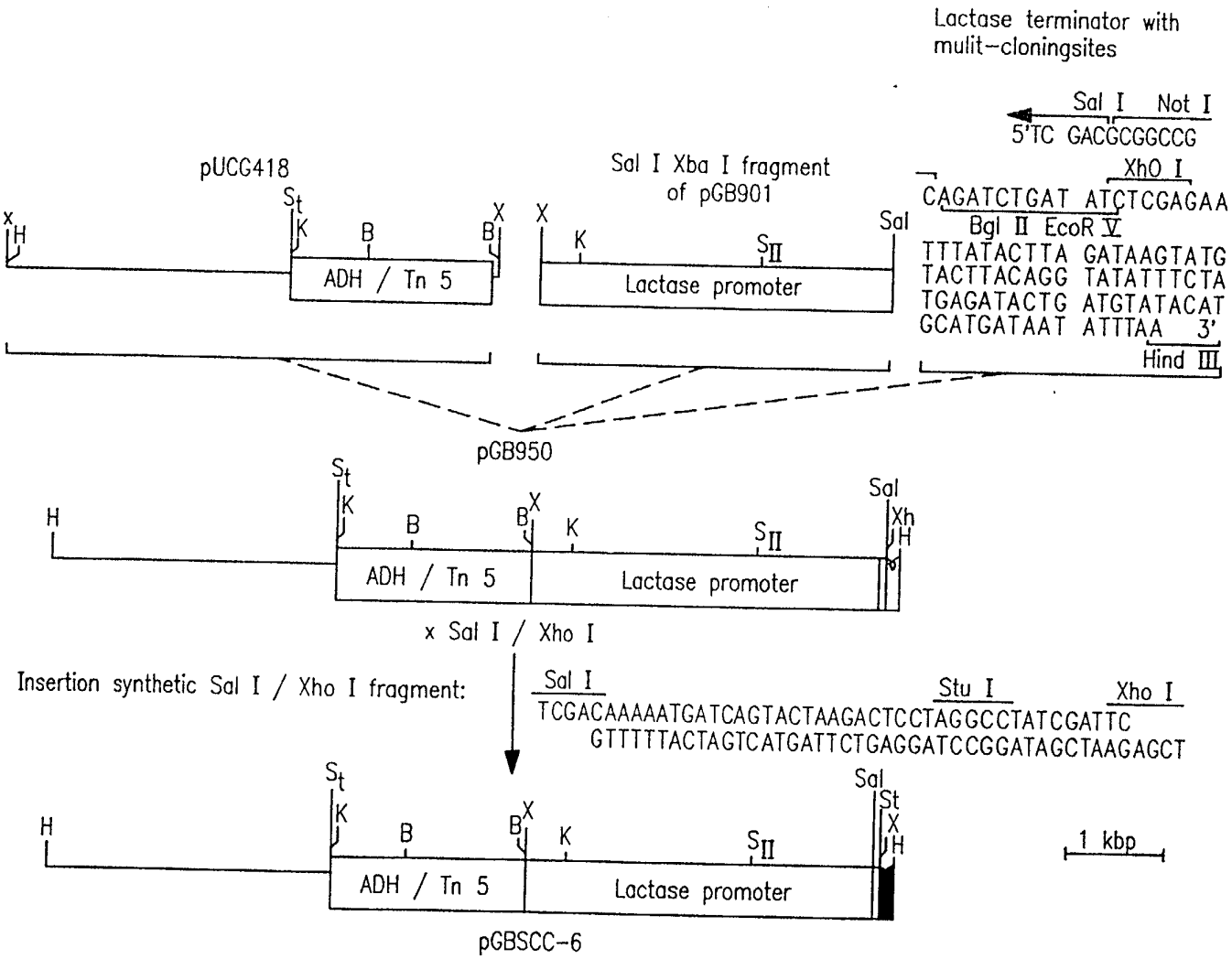

Lactase terminator with
mulit-cloningsites
Sal I Not I
5'TC GACGCGGCCG
XhO I
CAGATCTGAT ATCTCGAGAA
Bgl II EcoR V
TTTATACTTA GATAAGTATG
TACTTACAGG TATATTTCTA
TGAGATACTG ATGTATACAT
GCATGATAAT ATTTAA 3'
Hind III
pUC418
Sal I Xba I fragment
of pGB901
ADH / Tn 5
Lactase promoter
pGB950
x Sal I / Xho I
Insertion synthetic Sal I / Xho I fragment:
Sal I
Stu I
Xho I
TCGACAAAAATGATCAGTACTAAGACTCCTAGGCCTATCGATTC
GTTTTTACTAGTCATGATTCTGAGGATCCGGATAGCTAAGAGCT
1 kbp
pGBSCC-6

FIG. 13

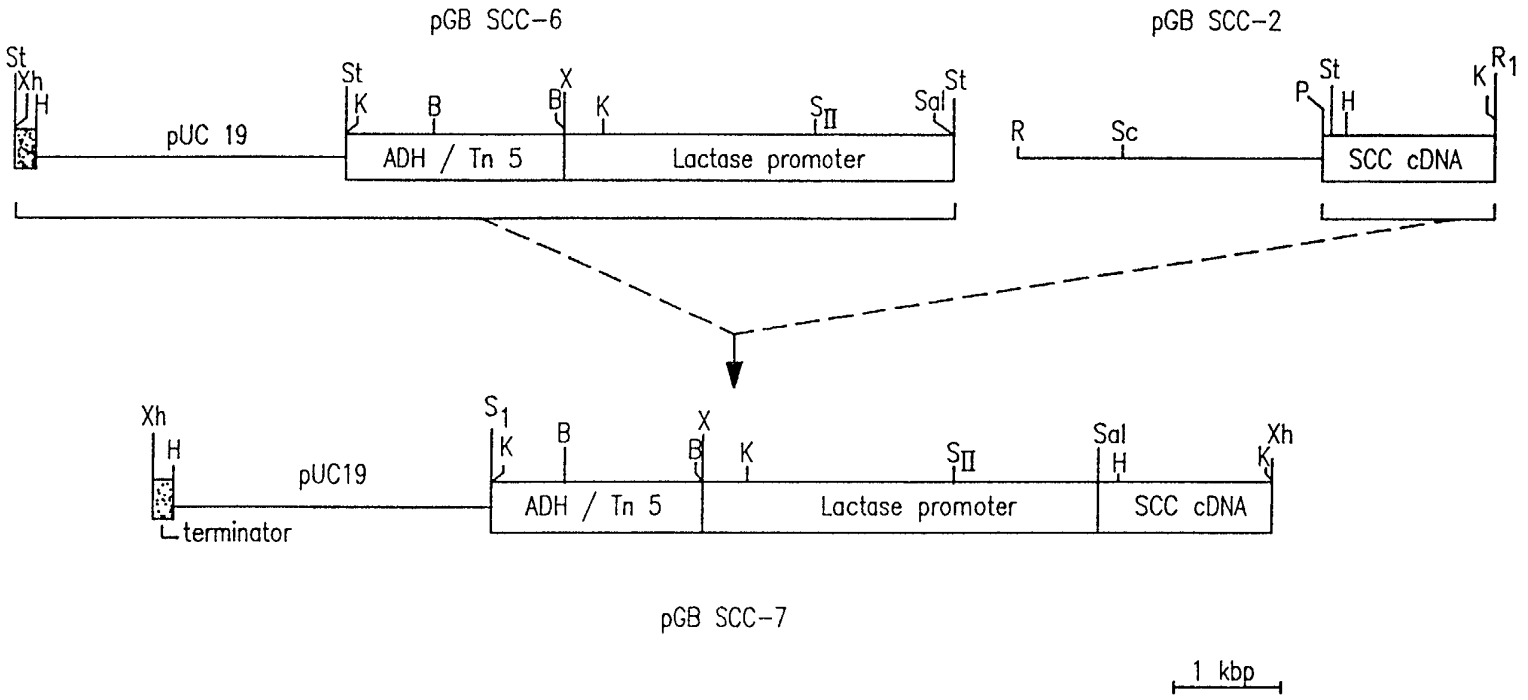


FIG. 14

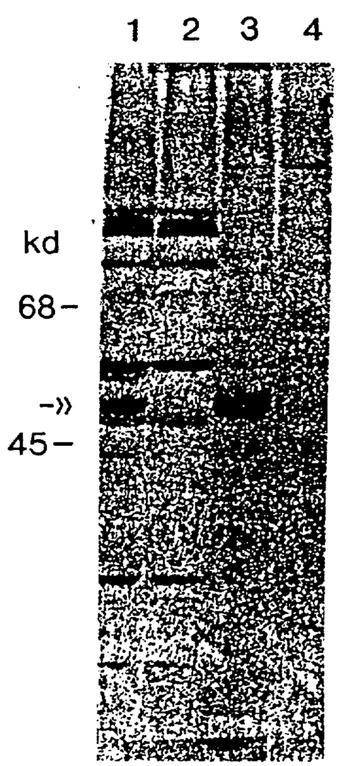


FIG. 15A

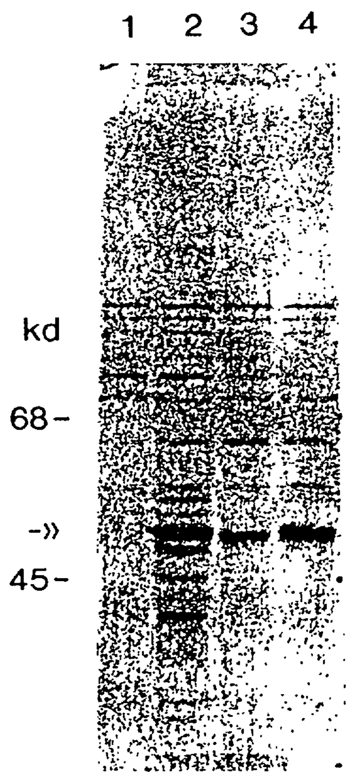


FIG. 15B

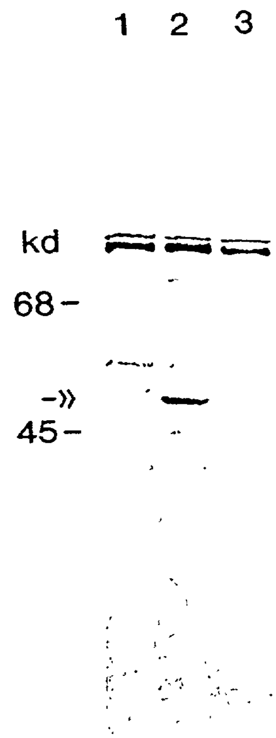


FIG. 15C

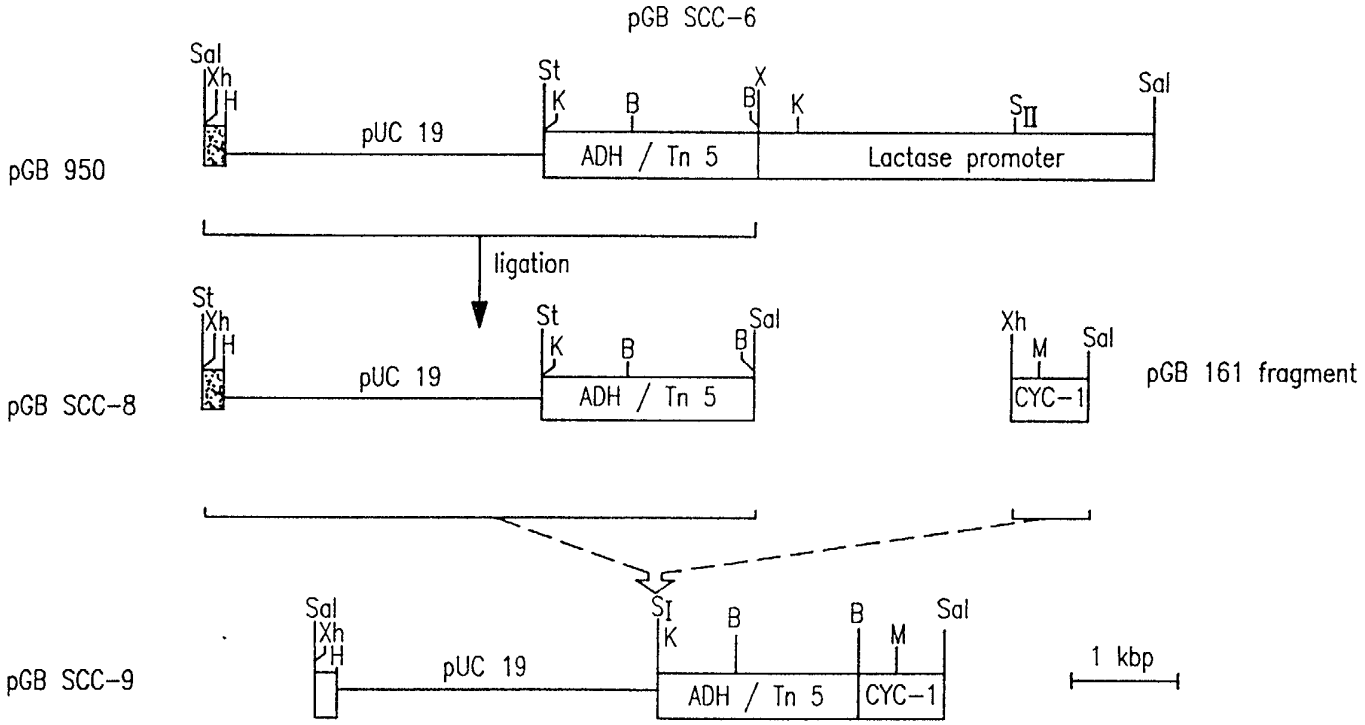
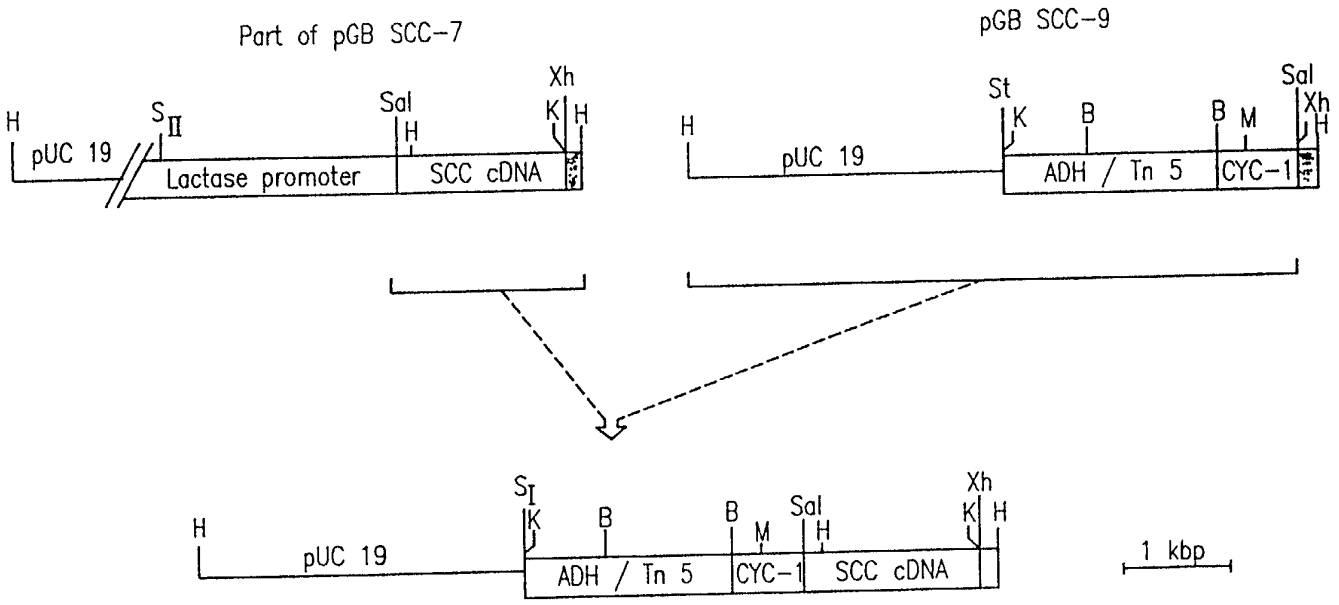


FIG. 16



pGB SCC-10

FIG. 17

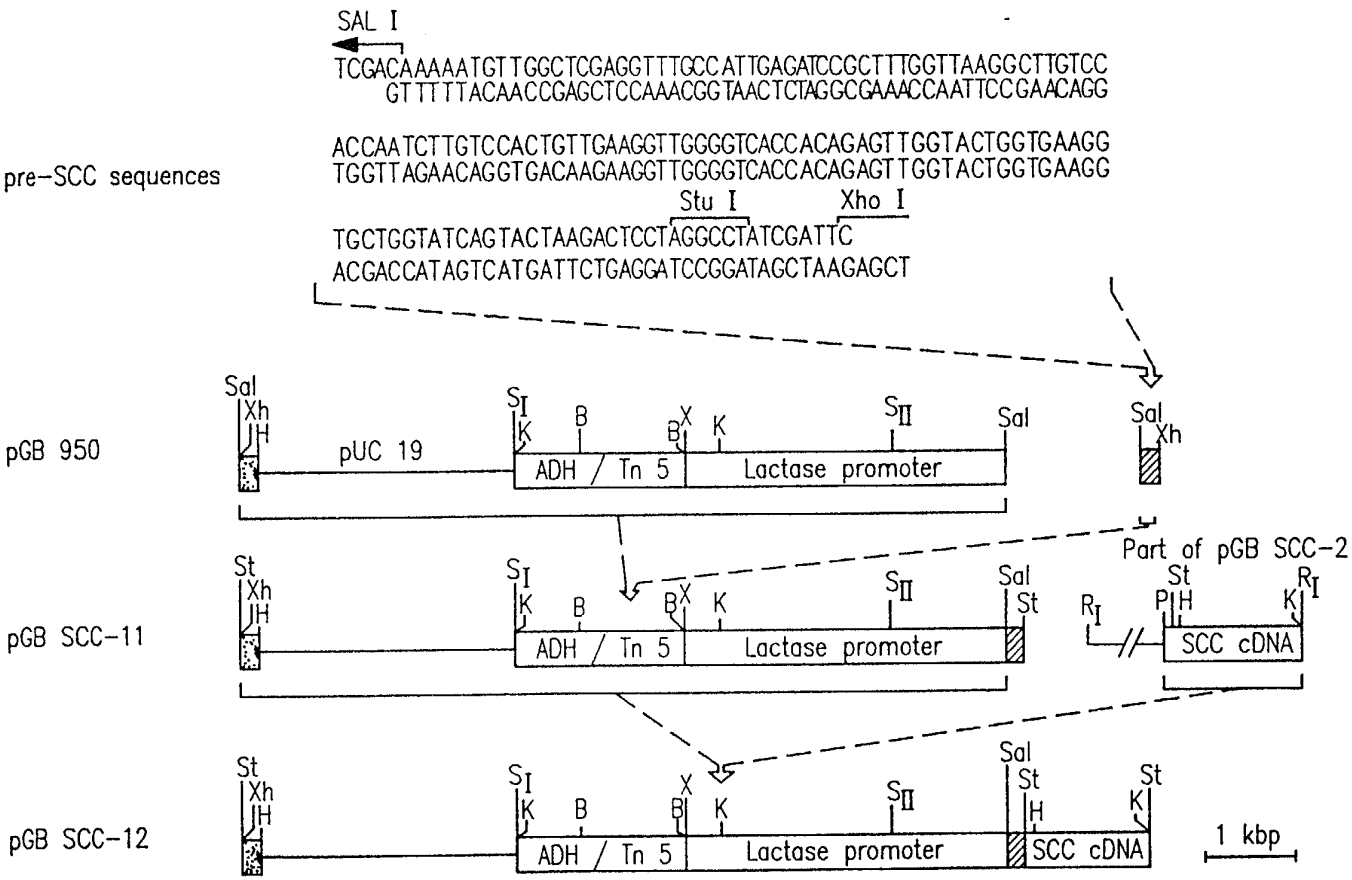
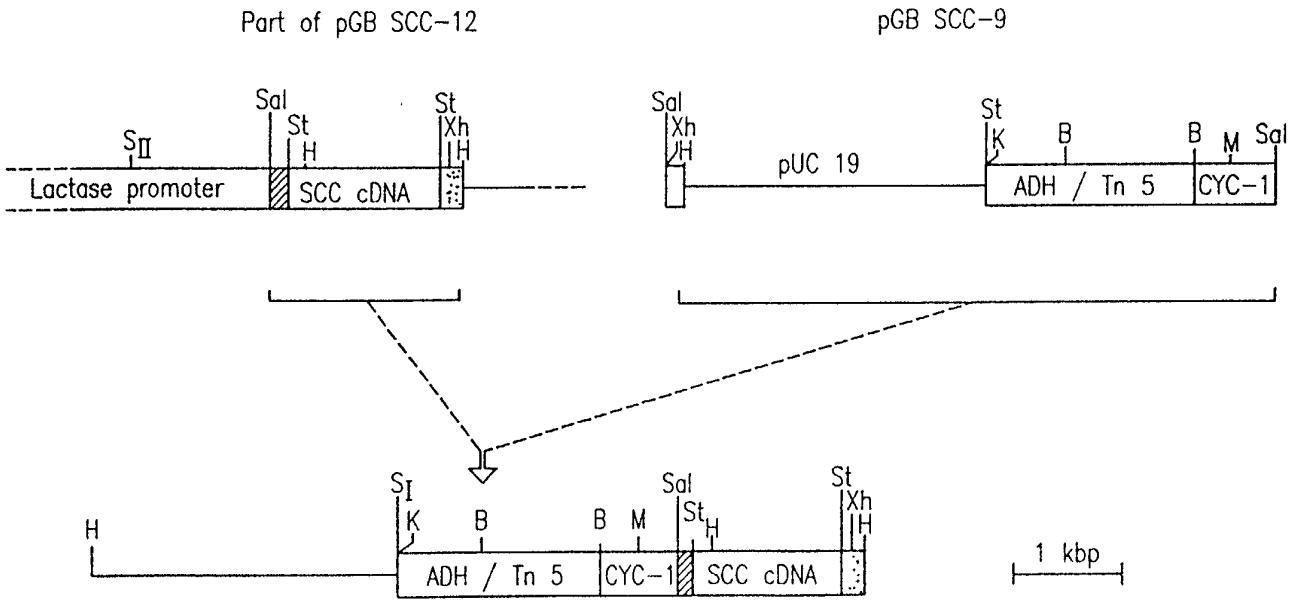

SAL I
TCGACAAAAATGTTGGCTCGAGGTTTGCCATTGAGATCCGCTTTGGTTAAGGCTTGTCC
GTTTTTACAACCGAGCTCCAAACGGTAACTCTAGGCGAAACCAATTCCGAACAGG
ACCAATCTTGTCCACTGTTGAAGGTTGGGGTCACCACAGAGTTGGTACTGGTGAAGG
TGGTTAGAACAGGTGACAAGAAGGTTGGGGTCACCACAGAGTTGGTACTGGTGAAGG
pre-SCC sequences
Stu I
Xho I
TGCTGGTATCAGTACTAAGACTCCTAGGCCTATCGATTC
ACGACCATAGTCATGATTCTGAGGATCCGGATAGCTAAGAGCT
pGB 950
pUC 19
ADH / Tn 5
Lactase promoter
pGB SCC-11
Part of pGB SCC-2
SCC cDNA
pGB SCC-12
1 kbp

FIG. 18



pGB SCC-13

FIG. 19

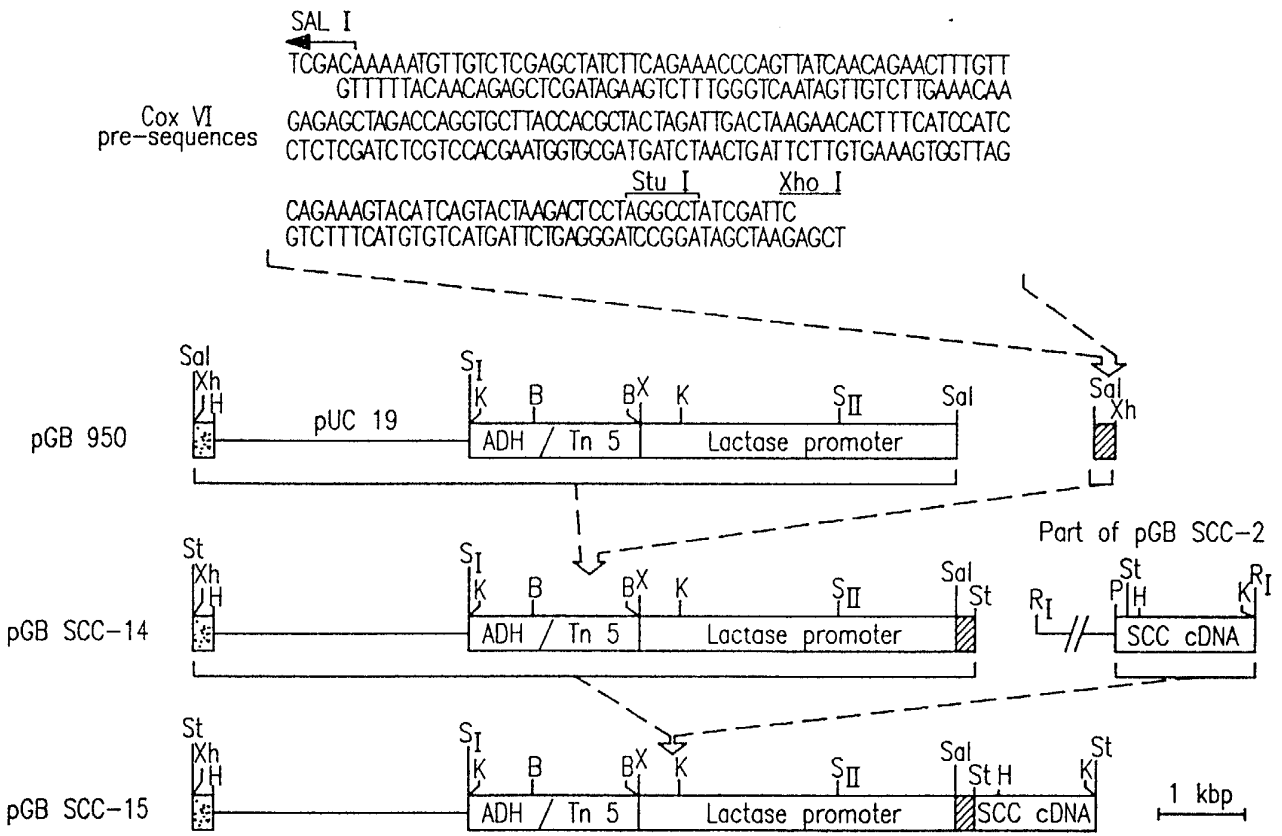


FIG. 20

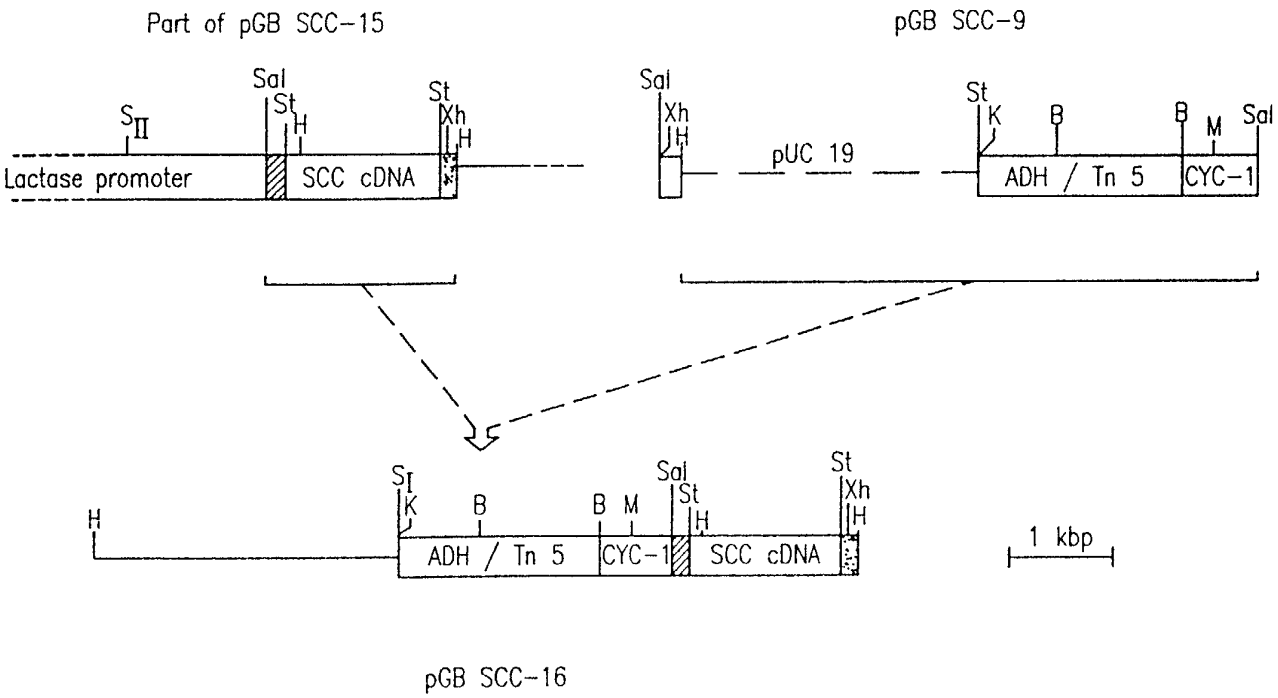

Part of pGB SCC-15
pGB SCC-9
SII
SaI
St
H
St
Xh
H
Lactase promoter
SCC cDNA
SaI
Xh
H
pUC 19
St
K
B
B
M
SaI
ADH / Tn 5
CYC-1
SI
K
B
B
M
SaI
St
H
St
Xh
H
H
ADH / Tn 5
CYC-1
SCC cDNA
1 kbp
pGB SCC-16

FIG. 21

FIG. 22A

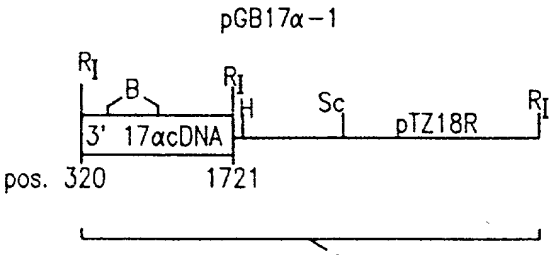
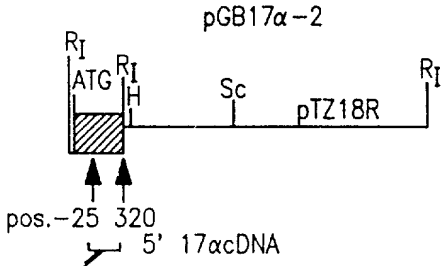
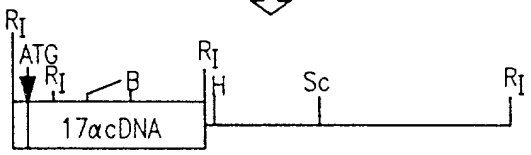


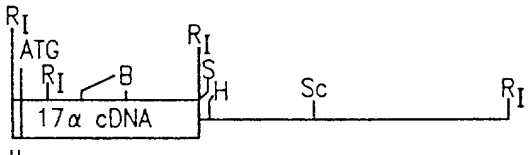
FIG. 22B





pGB17α-3

FIG. 22C



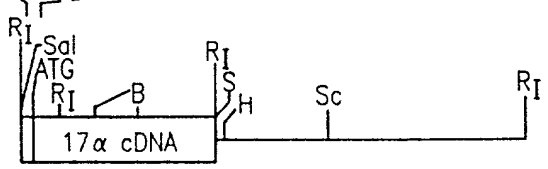
pGB17α-3

5'-TCTTTGTCCTGACTGCTGCCACCCAGACACAATGTGGCTGCTC-3'

In vitro mutagenesis

SAL I

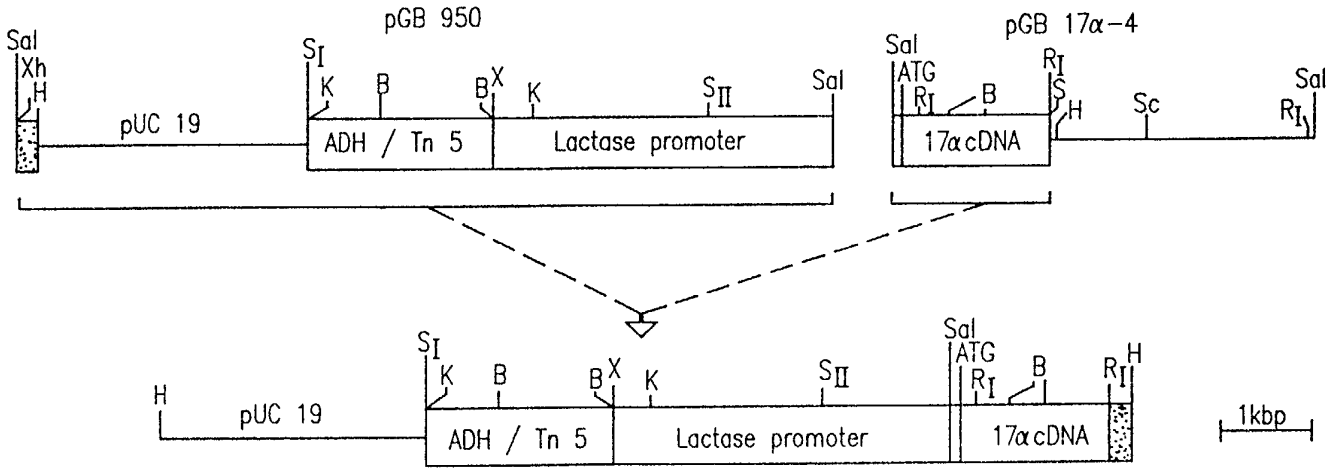
5'-TCTTTGTCCTGACTGCTGCCAGTCGACAAAAATGTGGCTGCTC-3'



pGB17α-4

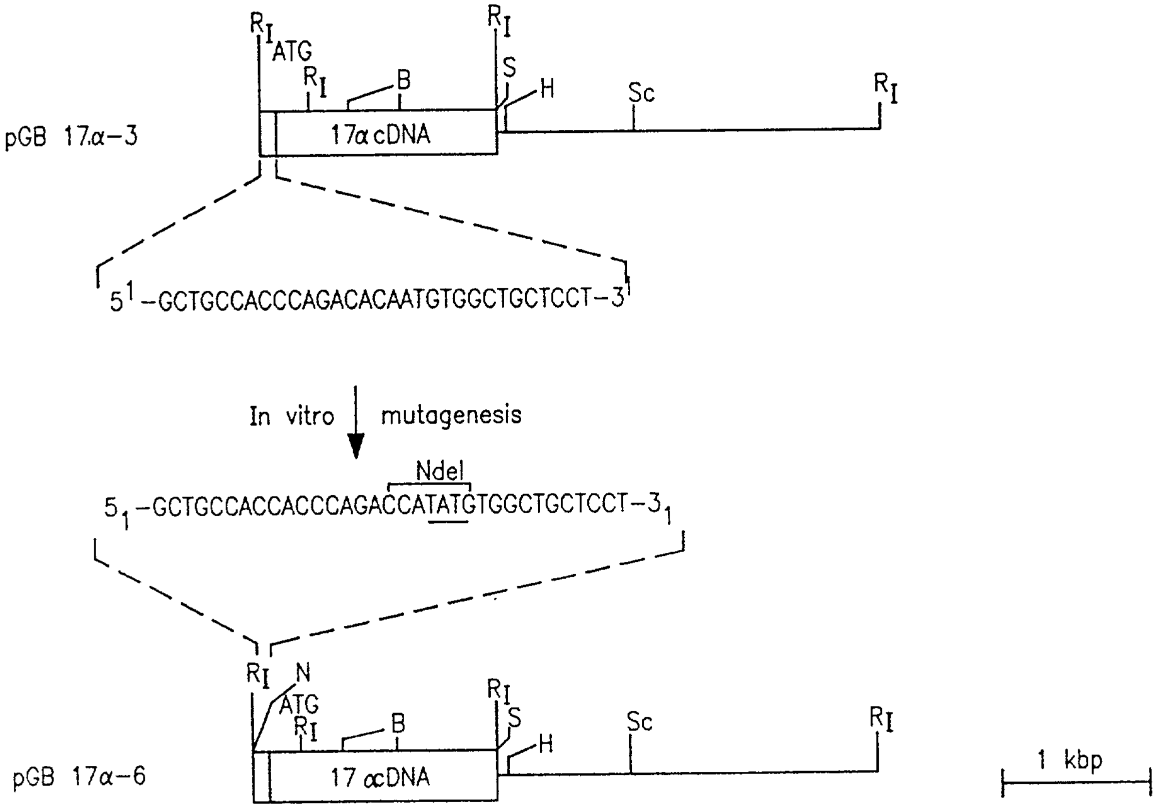
1 kbp

FIG. 23



pGB 17α-5

FIG. 24



pGB 17α-3
RI
ATG
RI
B
RI
S
H
Sc
RI
17α cDNA
5' -GCTGCCACCCAGACACAATGTGGCTGCTCCT-3'
In vitro mutagenesis
NdeI
5' -GCTGCCACCACCCAGACCATATGTGGCTGCTCCT-3'
pGB 17α-6
RI
N
ATG
RI
B
RI
S
H
Sc
RI
17 αcDNA
1 kbp

FIG. 25

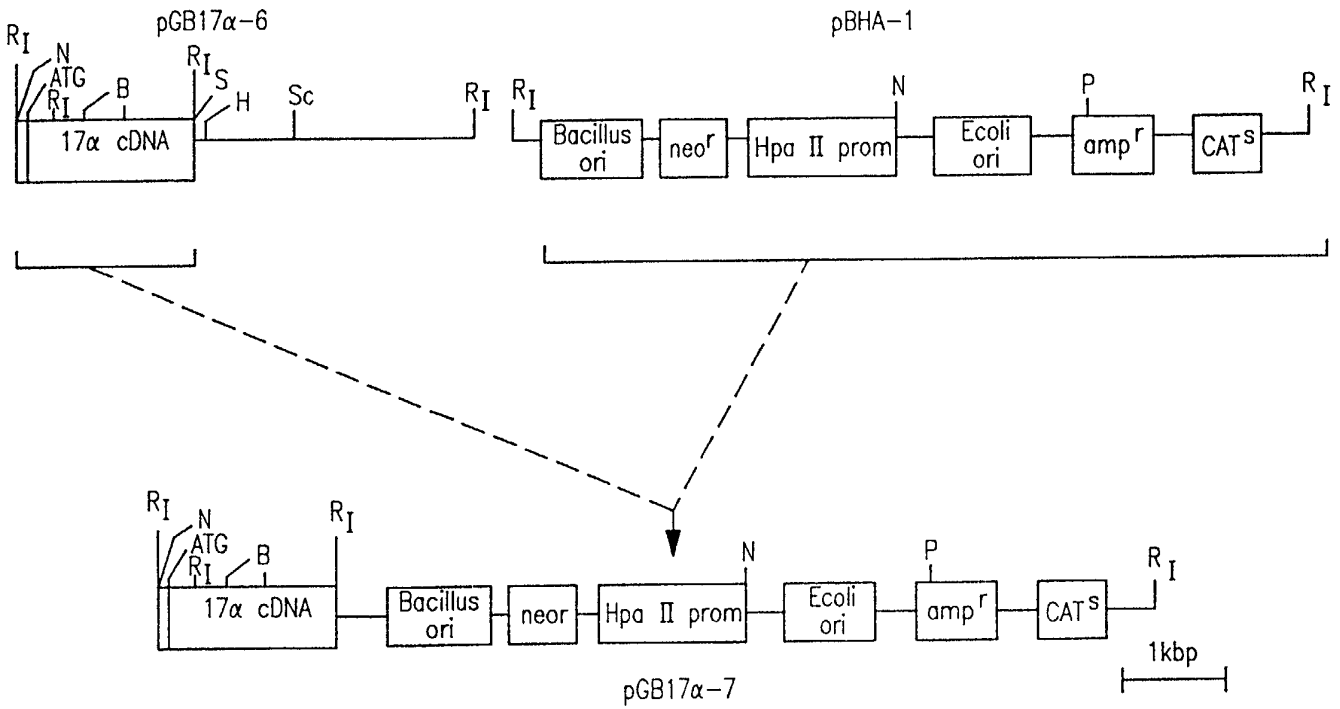

pGB17α-6
pBHA-1
RI
N
ATG
RI
B
RI
S
H
Sc
RI
17α cDNA
RI
Bacillus ori
neor
Hpa II prom
N
Ecoli ori
P
ampr
CATs
RI
RI
N
ATG
RI
B
RI
17α cDNA
Bacillus ori
neor
Hpa II prom
N
Ecoli ori
P
ampr
CATs
RI
1kbp
pGB17α-7

FIG. 26

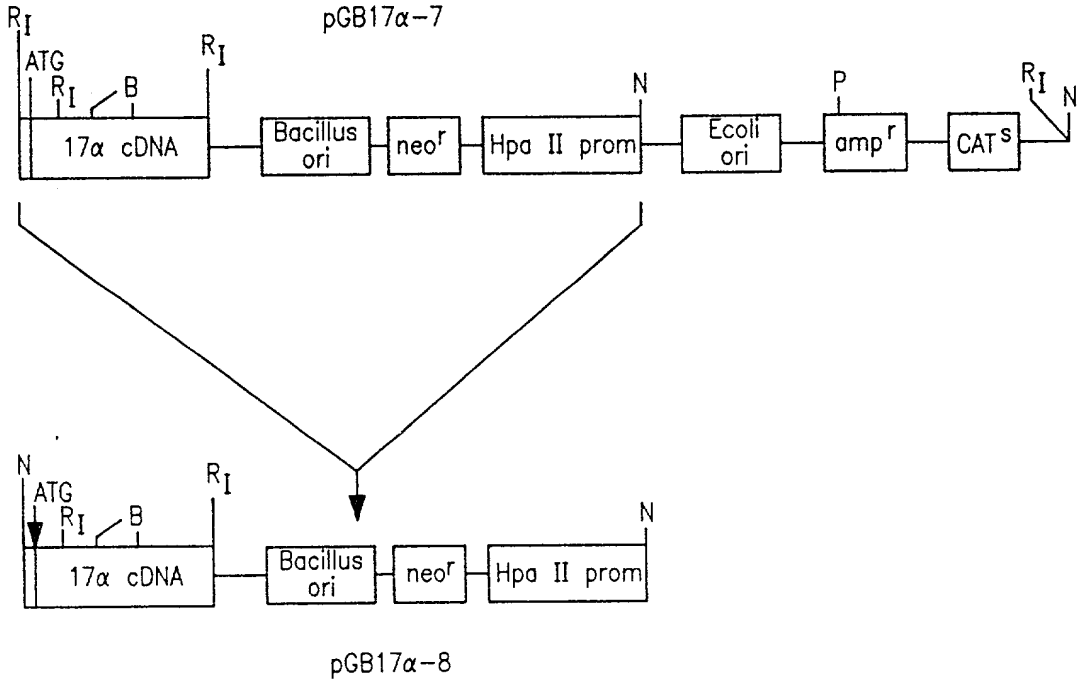

pGB17α-7
ATG
B
17α cDNA
Bacillus ori
neo^r
Hpa II prom
Ecoli ori
amp^r
CAT^s
pGB17α-8

FIG. 27

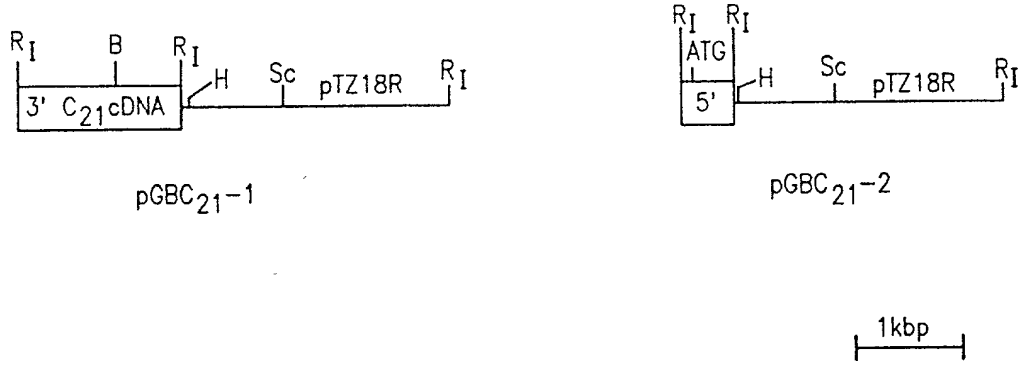

B
3' C21cDNA
H
Sc
pTZ18R
pGBC21-1
ATG
5'
pGBC21-2
1kbp

FIG. 28

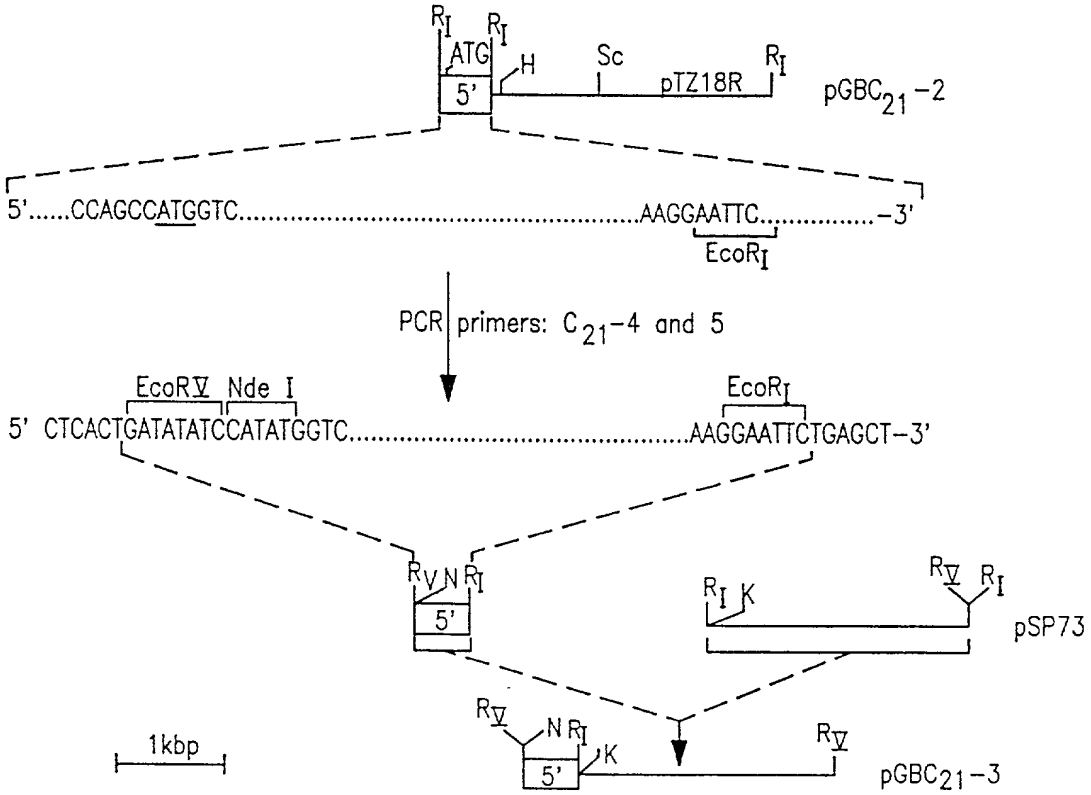


FIG. 29

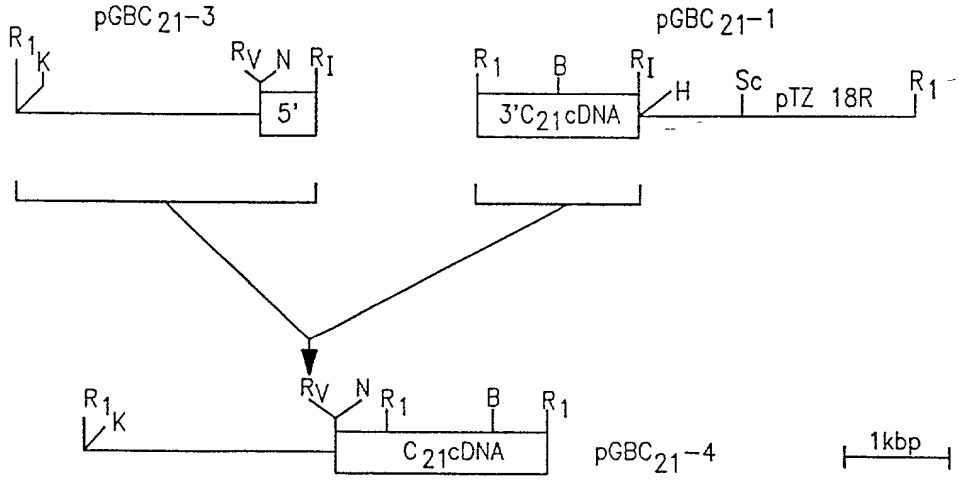


FIG. 30

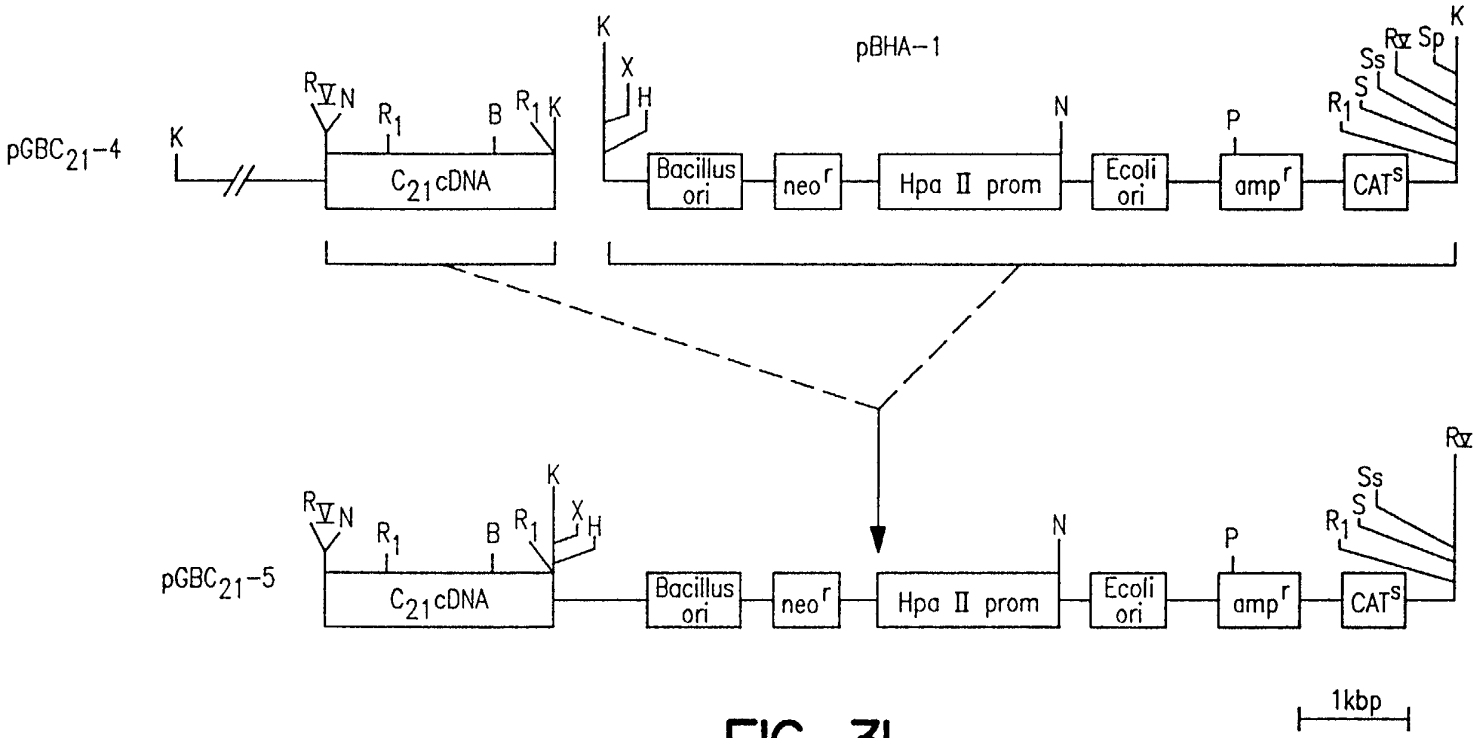

pGBC21-4
K
RV N
R1
B
R1 K
C21 cDNA
K
X
H
pBHA-1
Bacillus ori
neor
Hpa II prom
N
Ecoli ori
P
ampr
CATs
R1
S
Ss
RV
Sp
K
pGBC21-5
K
X
H
RV
1kbp

FIG. 31

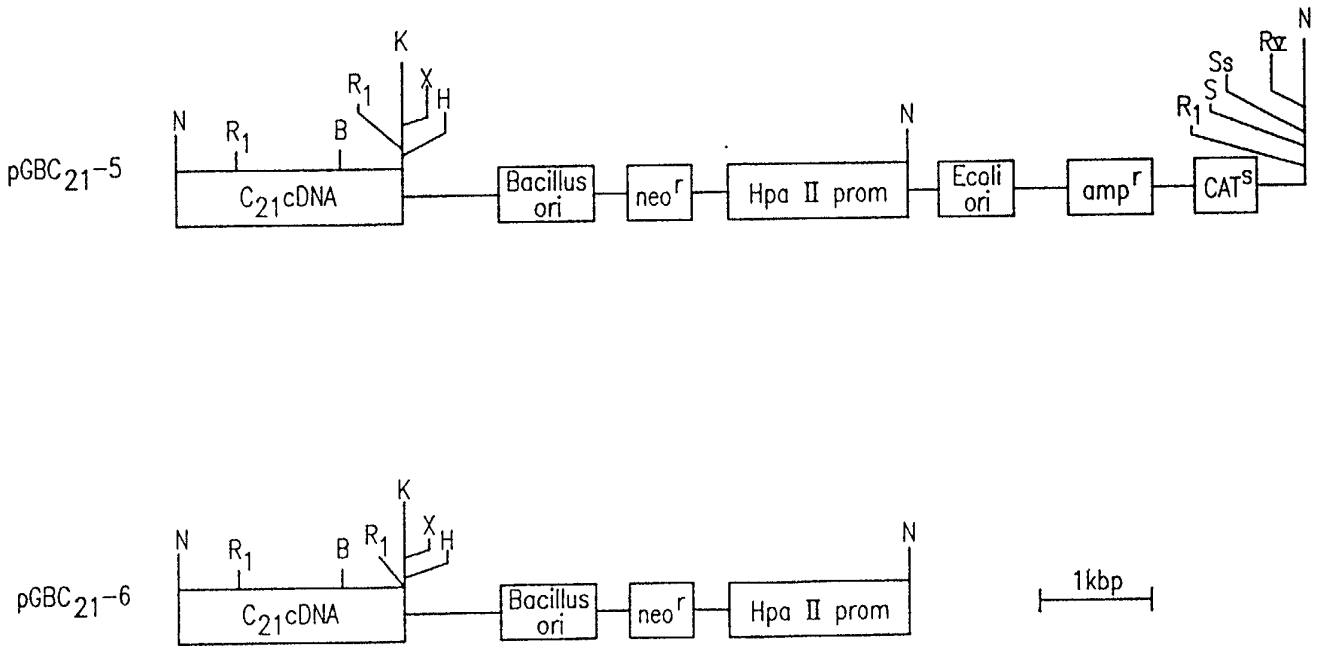

pGBC21-5
N
R1
B
K
R1
X
H
C21cDNA
Bacillus ori
neor
N
Hpa II prom
Ecoli ori
ampr
R1
S
Ss
Rv
N
CATs
pGBC21-6
N
R1
B
K
R1
X
H
C21cDNA
Bacillus ori
neor
N
Hpa II prom
1kbp

FIG. 32

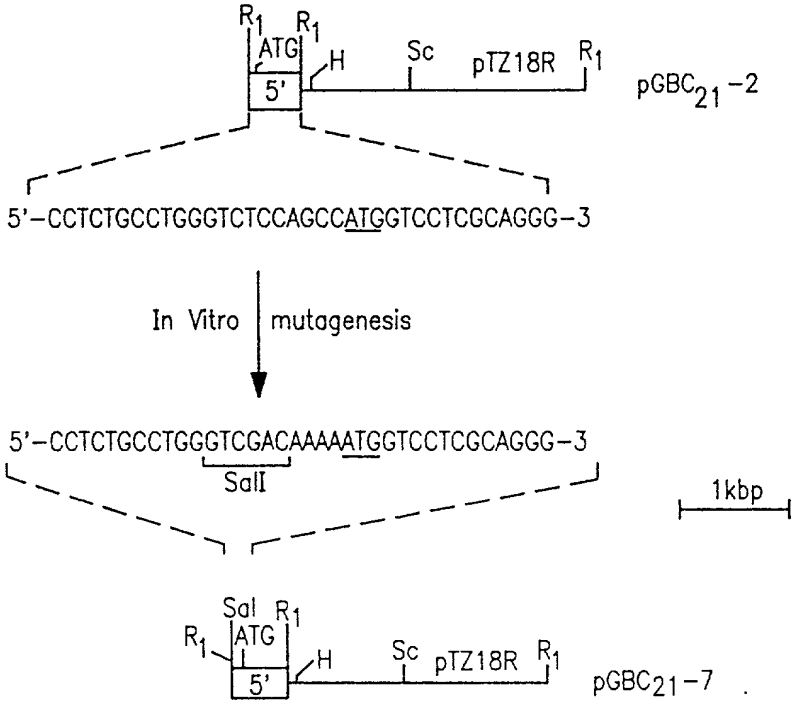

R1 R1
ATG
H
Sc
pTZ18R
R1
5'
pGBC21-2
5'-CCTCTGCCTGGGTCTCCAGCCATGGTCCTCGCAGGG-3
In Vitro mutagenesis
5'-CCTCTGCCTGGGTCGACAAAAATGGTCCTCGCAGGG-3
SalI
1kbp
Sal R1
R1
ATG
H
Sc
pTZ18R
R1
5'
pGBC21-7

FIG. 33

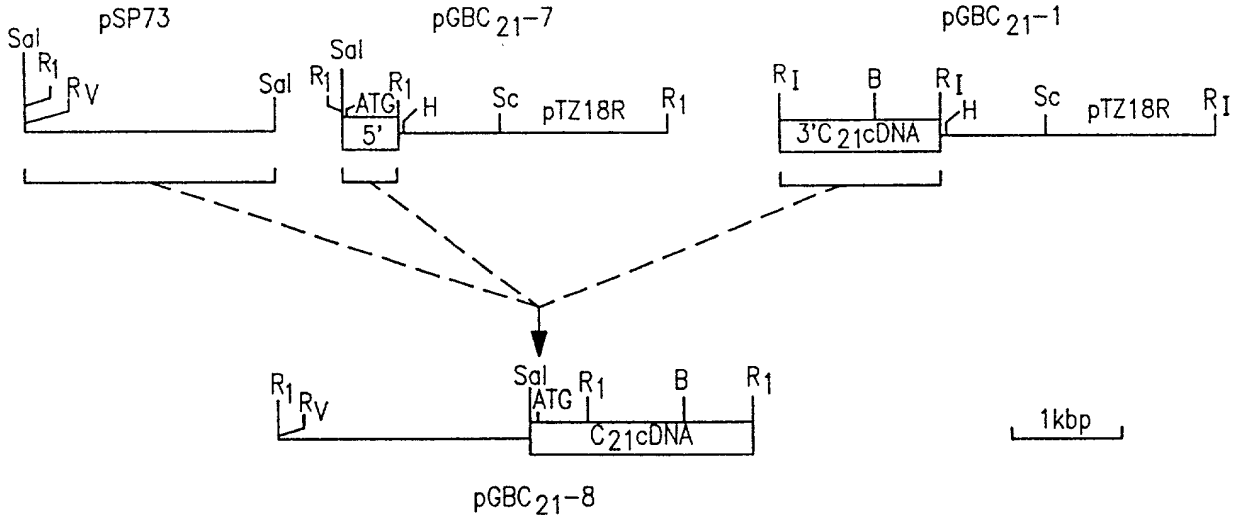

pSP73
pGBC21-7
pGBC21-1
Sal
R1
RV
Sal
Sal
R1
R1
ATG
H
Sc
pTZ18R
R1
5'
RI
B
RI
H
Sc
pTZ18R
RI
3'C21cDNA
R1
RV
Sal
ATG
R1
B
R1
C21cDNA
1kbp
pGBC21-8

FIG. 34

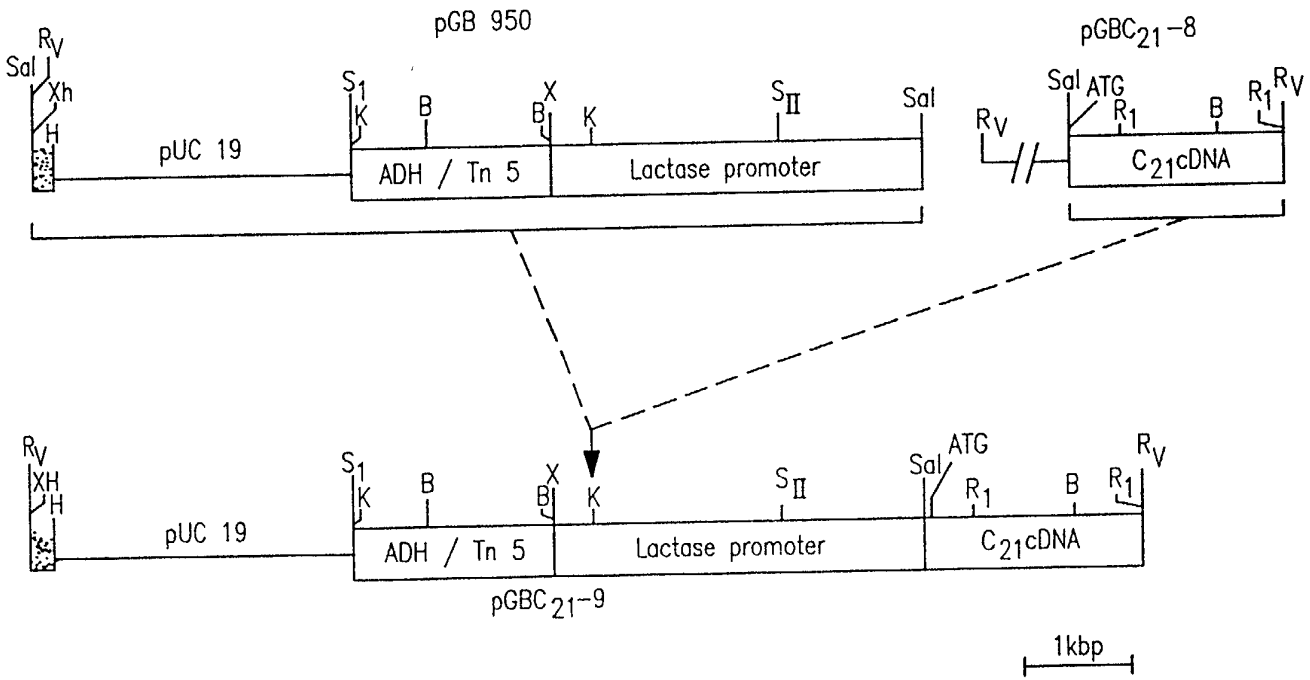


FIG. 35

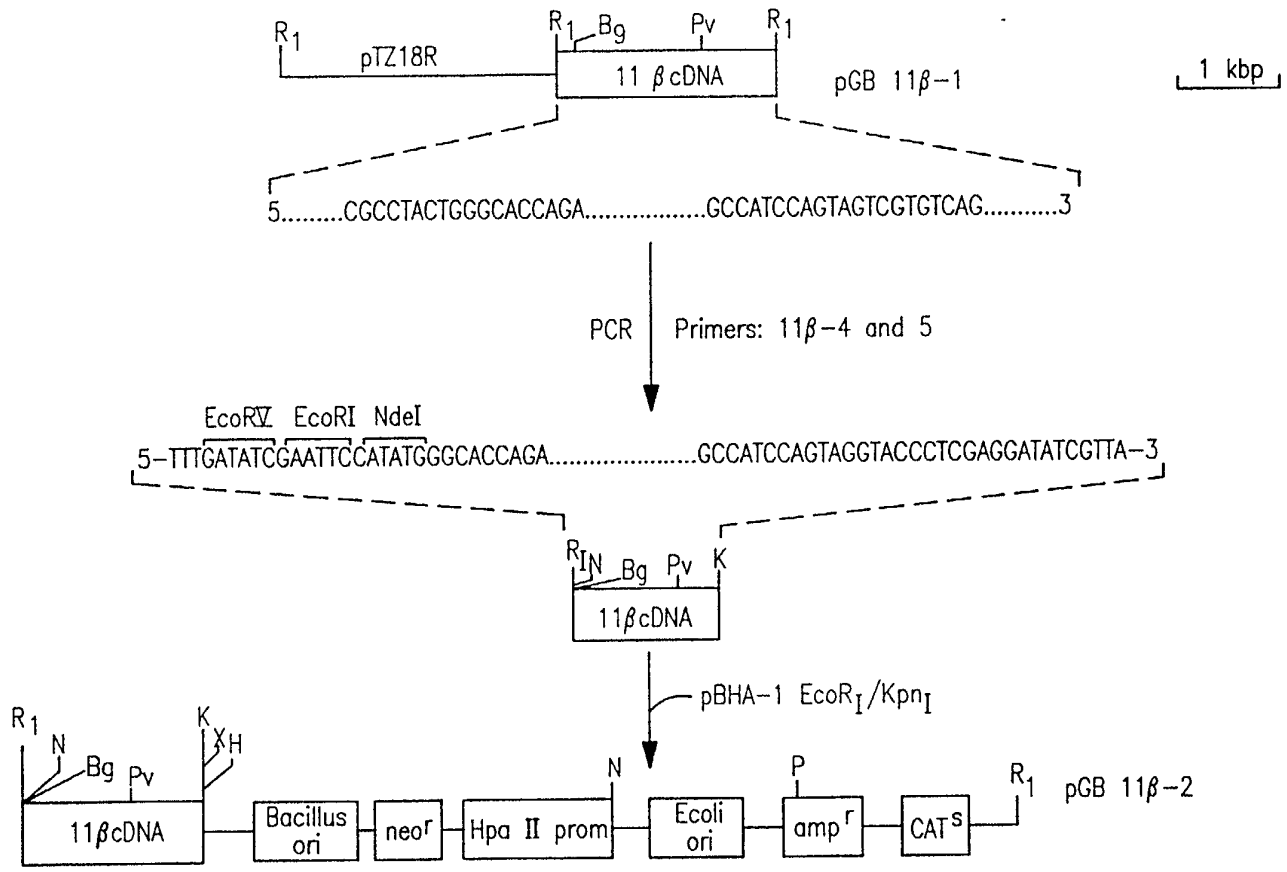

R1
pTZ18R
R1 Bg Pv R1
11 β cDNA
pGB 11β-1
1 kbp
5.....CGCCTACTGGGCACCAGA.....GCCATCCAGTAGTCGTGTCAG.....3
PCR
Primers: 11β-4 and 5
EcoRV EcoRI NdeI
5-TTTGATATCGAATTCCATATGGGCACCAGA.....GCCATCCAGTAGGTACCCTCGAGGATATCGTTA-3
R1 N Bg Pv K
11βcDNA
pBHA-1 EcoRI/KpnI
R1 N Bg Pv K X H
11βcDNA
Bacillus ori
neor
N
Hpa II prom
Ecoli ori
P
ampr
CATs
R1
pGB 11β-2

FIG. 36

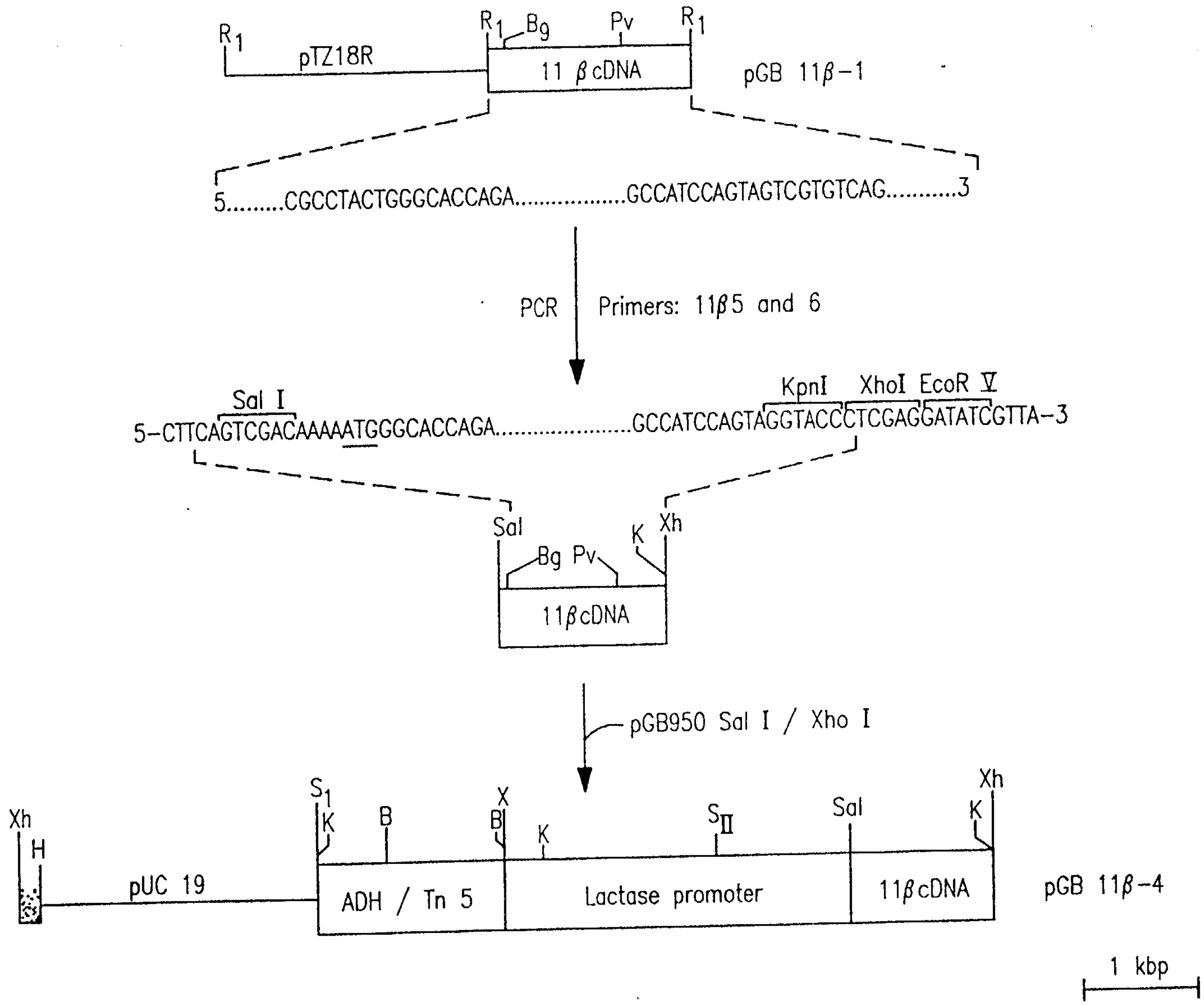
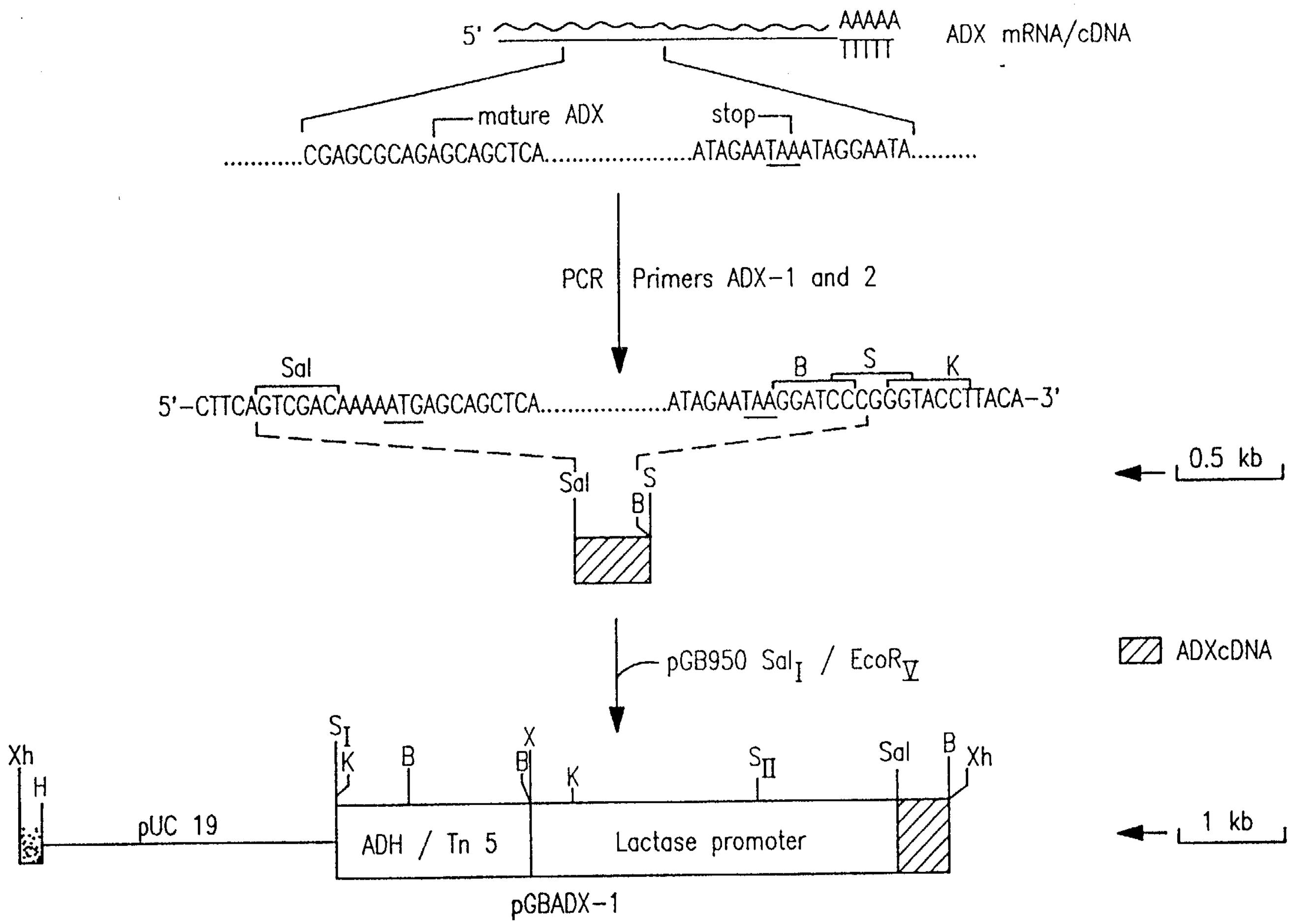

R1
pTZ18R
R1
Bg
Pv
R1
11 β cDNA
pGB 11β-1
5.........CGCCTACTGGGCACCAGA.................GCCATCCAGTAGTCGTGTCAG...........3
PCR
Primers: 11β5 and 6
Sal I
KpnI
XhoI
EcoR V
5-CTTCAGTCGACAAAAATGGGCACCAGA.....................GCCATCCAGTAGGTACCCTCGAGGATATCGTTA-3
Sal
K
Xh
Bg
Pv
11βcDNA
pGB950 Sal I / Xho I
Xh
H
pUC 19
S1
K
B
X
B
K
SII
Sal
K
Xh
ADH / Tn 5
Lactase promoter
11βcDNA
pGB 11β-4
1 kbp

FIG. 37



5'
AAAAA
TTTTT
ADX mRNA/cDNA
mature ADX
stop
..........CGAGCGCAGAGCAGCTCA....................ATAGAATAAATAGGAATA.........
PCR
Primers ADX-1 and 2
Sal
B
S
K
5'-CTTCAGTCGACAAAAATGAGCAGCTCA.................ATAGAATAAGGATCCCGGGTACCTTACA-3'
Sal
S
B
0.5 kb
pGB950 Sal$_I$ / EcoR$_V$
ADXcDNA
Xh
H
pUC 19
S$_I$
K
B
X
B
K
S$_{II}$
Sal
B
Xh
ADH / Tn 5
Lactase promoter
pGBADX-1
1 kb

FIG. 38

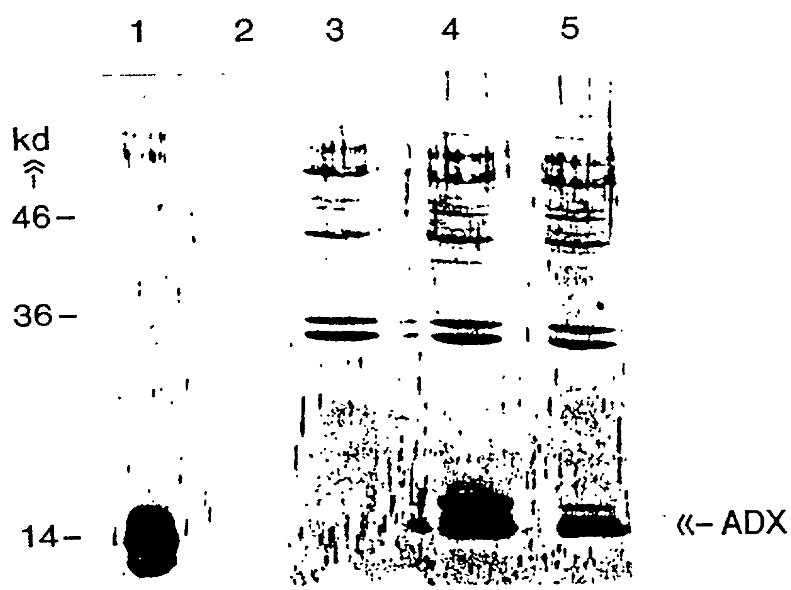


FIG. 39

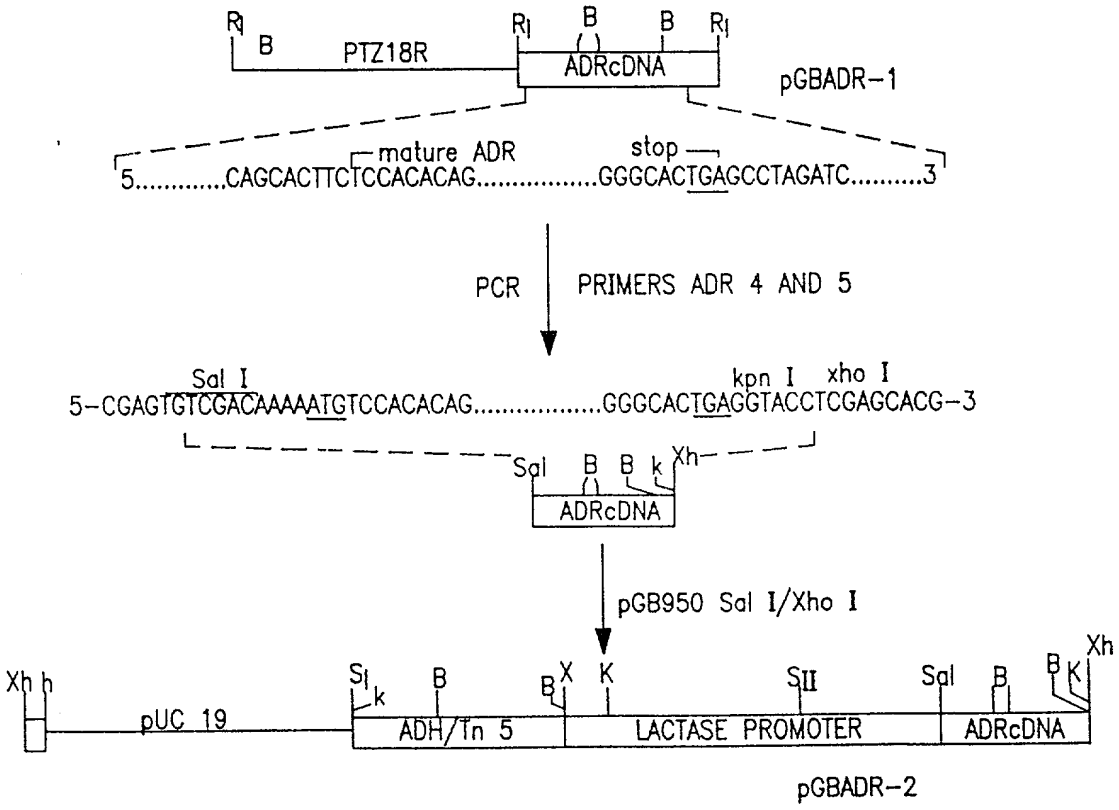
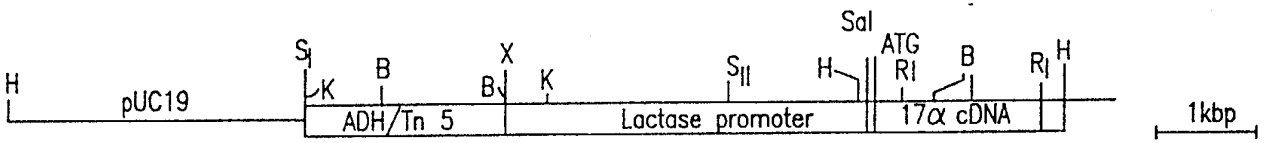


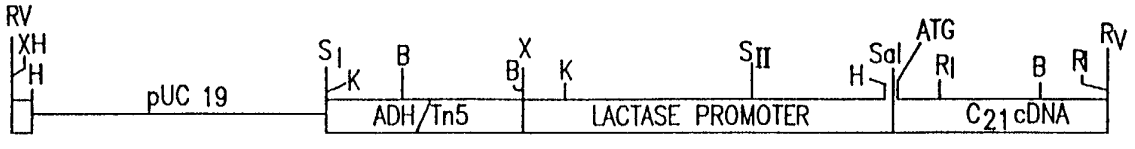
FIG. 40



pGB 17α-5

Lactase terminator

FIG. 41



pGBC 21-9

Lactase terminator

FIG. 42

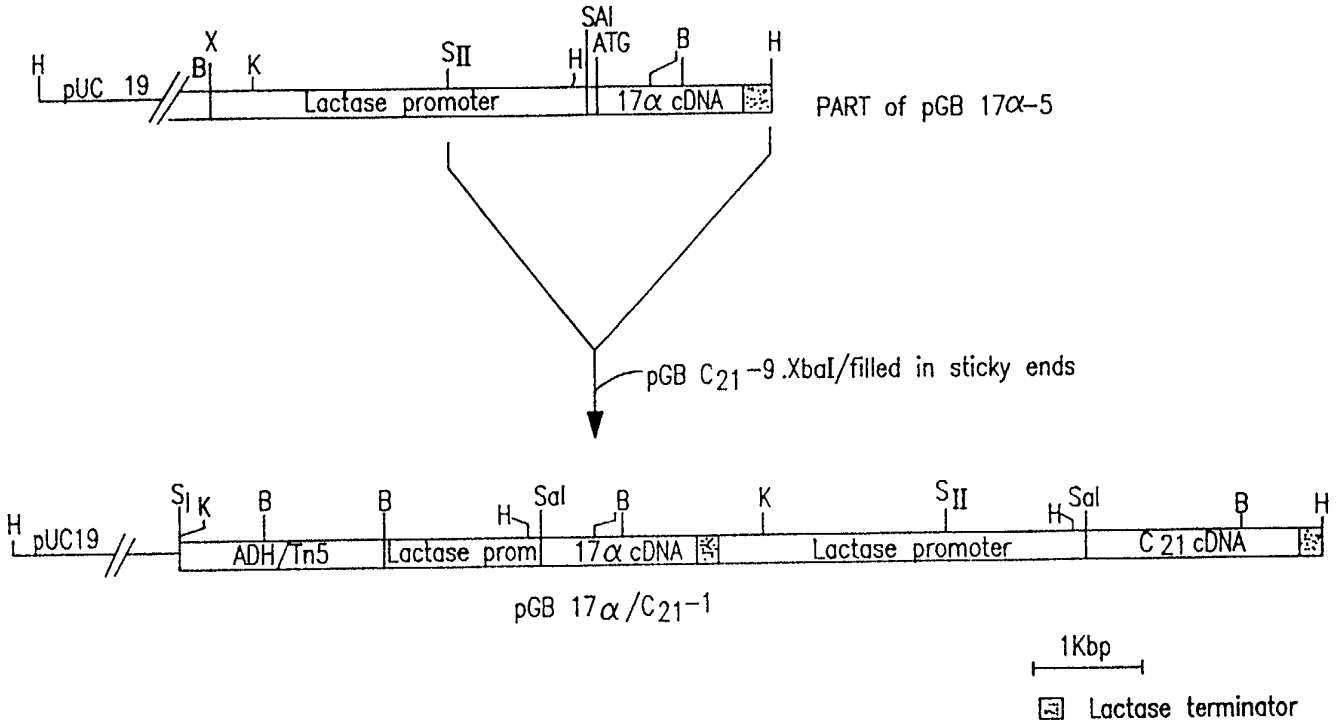


FIG. 43

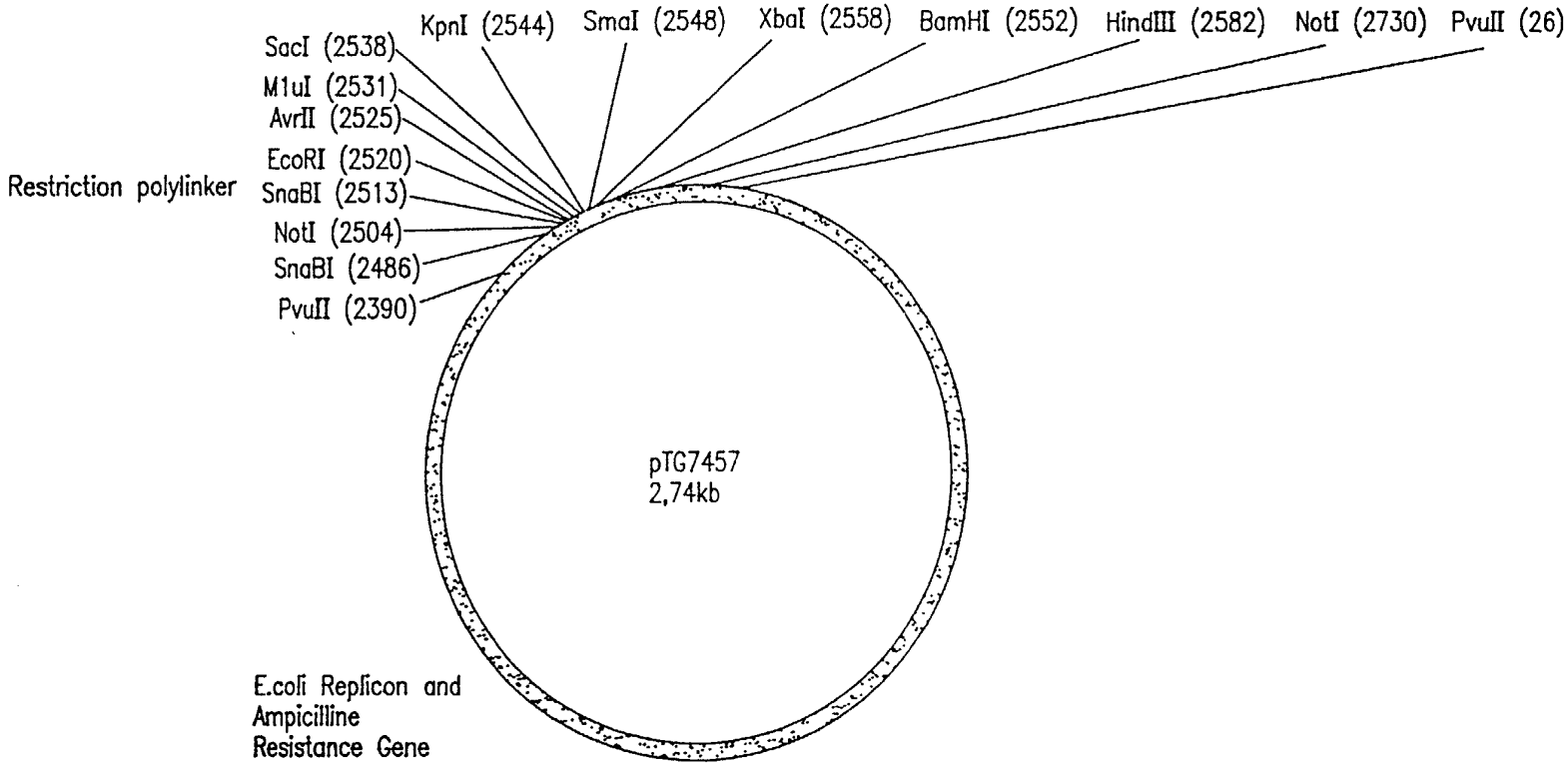

KpnI (2544)
SmaI (2548)
XbaI (2558)
BamHI (2552)
HindIII (2582)
NotI (2730)
PvuII (26)
SacI (2538)
M1uI (2531)
AvrII (2525)
EcoRI (2520)
SnaBI (2513)
NotI (2504)
SnaBI (2486)
PvuII (2390)
Restriction polylinker
pTG7457
2,74kb
E.coli Replicon and
Ampicilline
Resistance Gene

FIG. 44

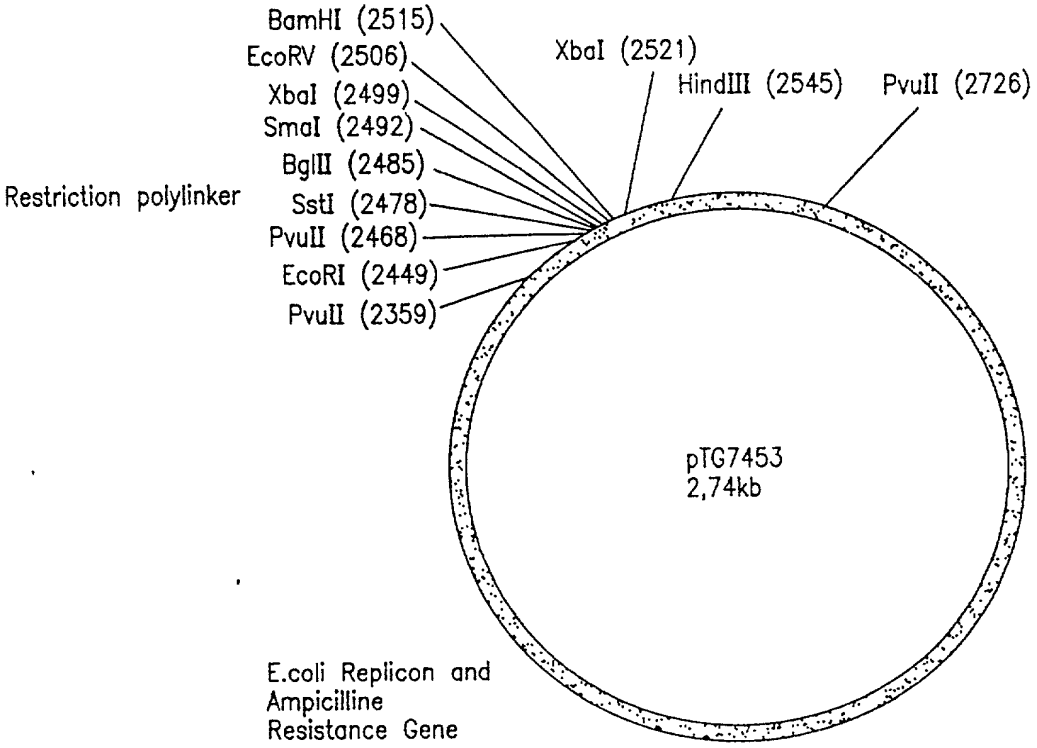


FIG. 45

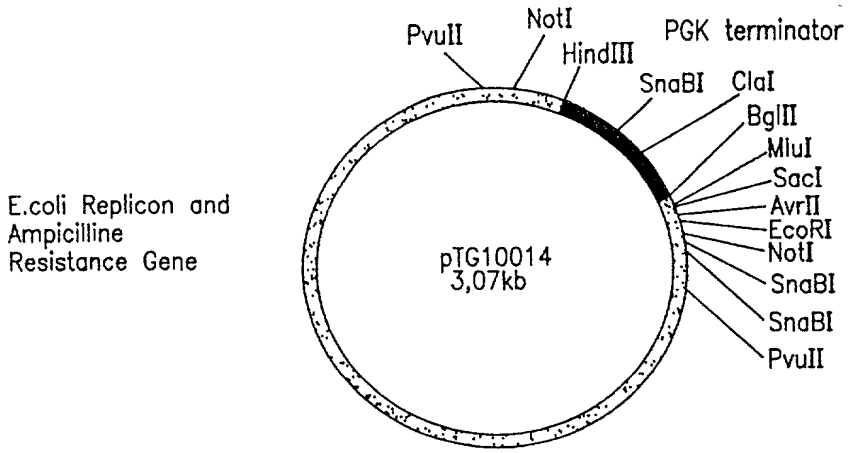


FIG. 46

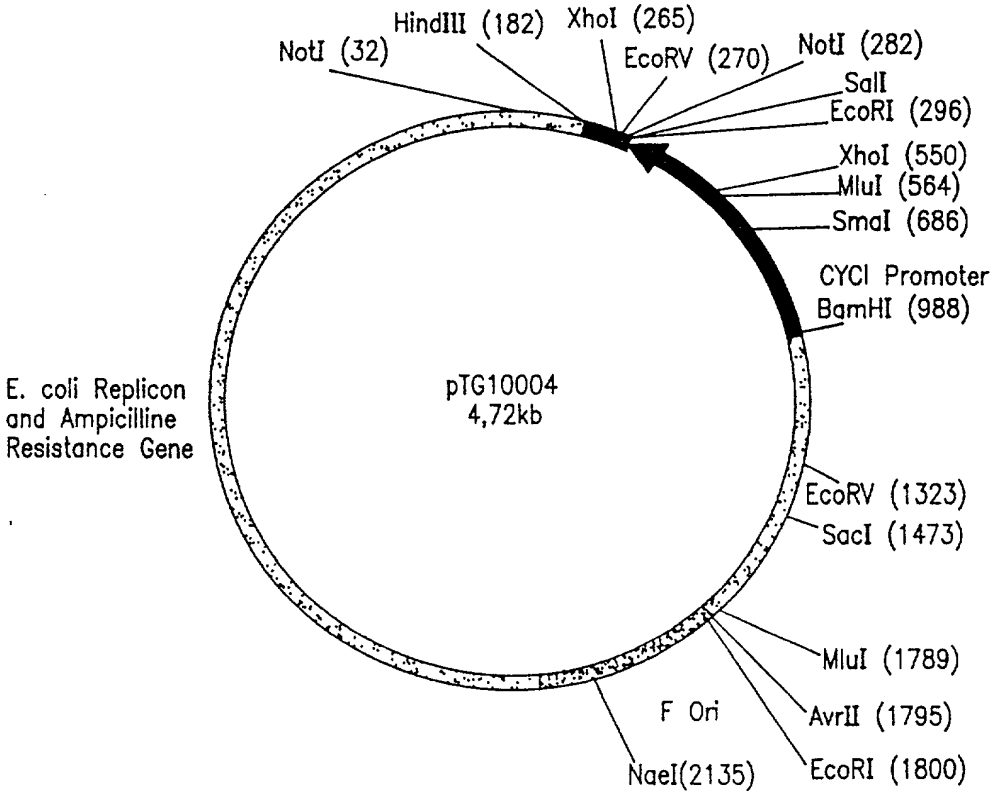


FIG. 47

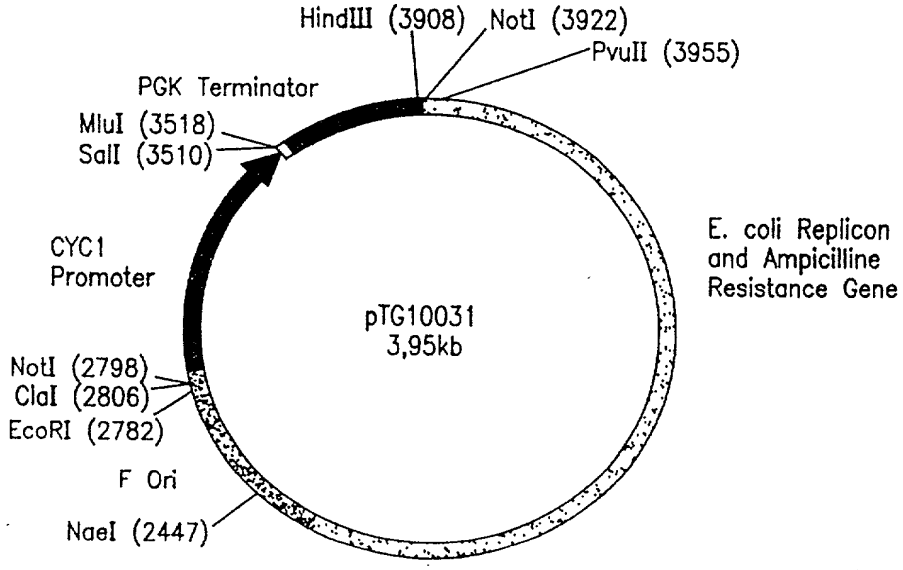


FIG. 48

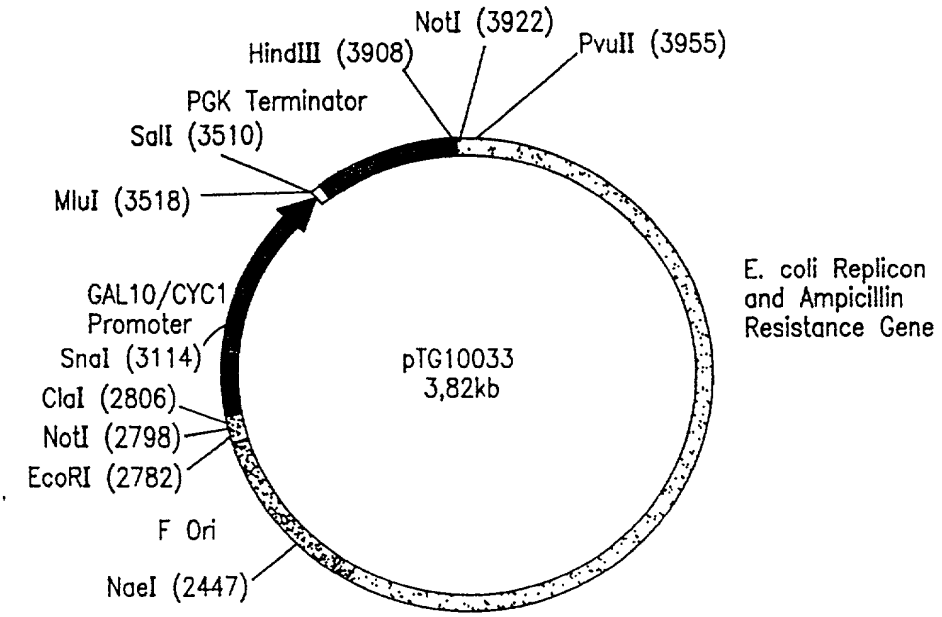


FIG. 49

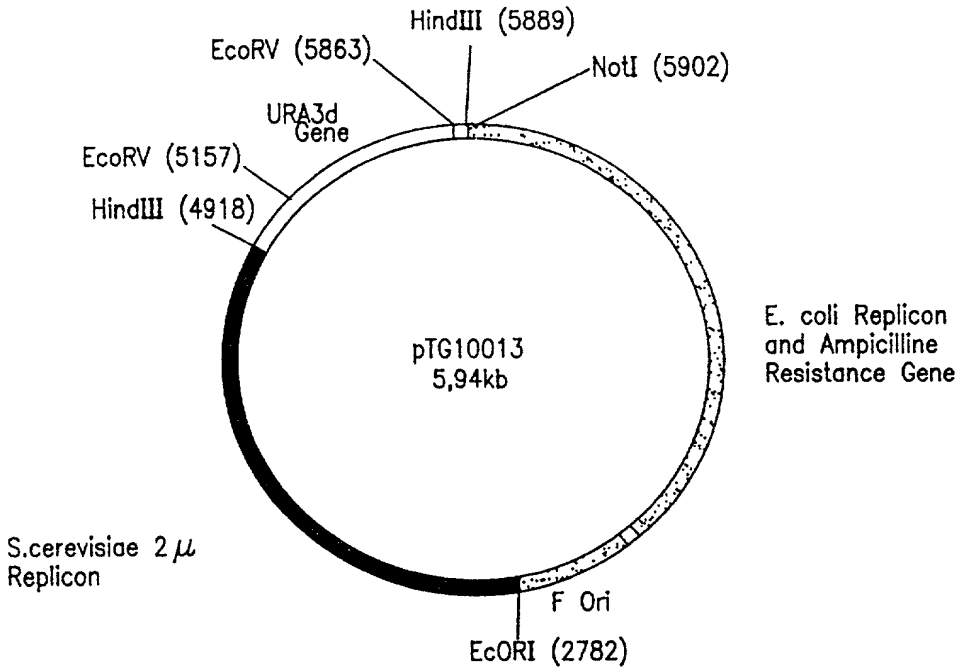


FIG. 50

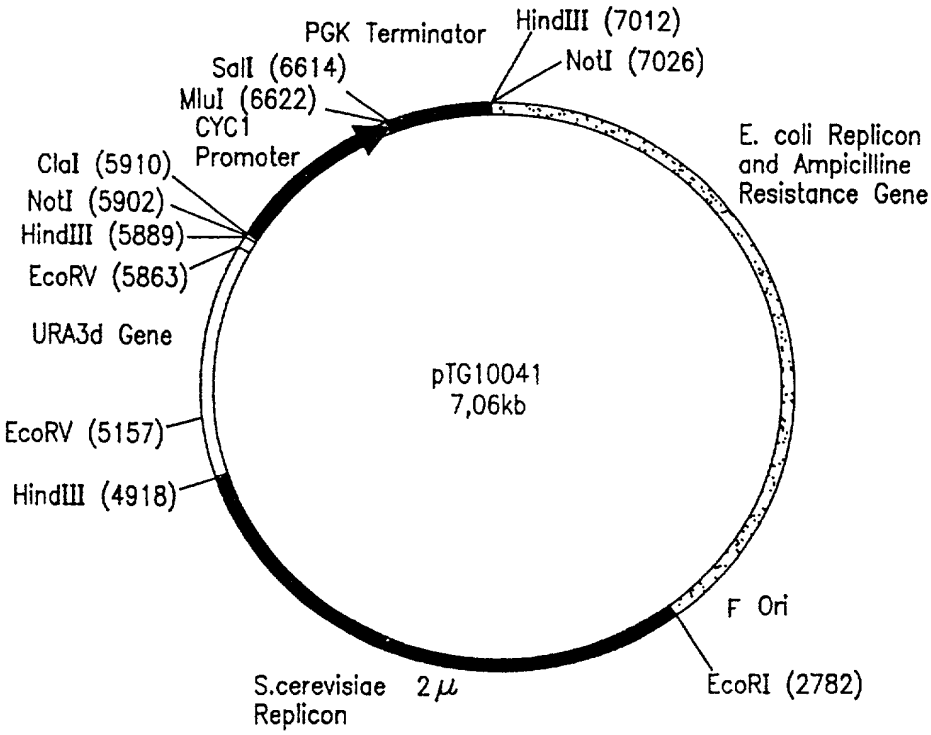


FIG. 51

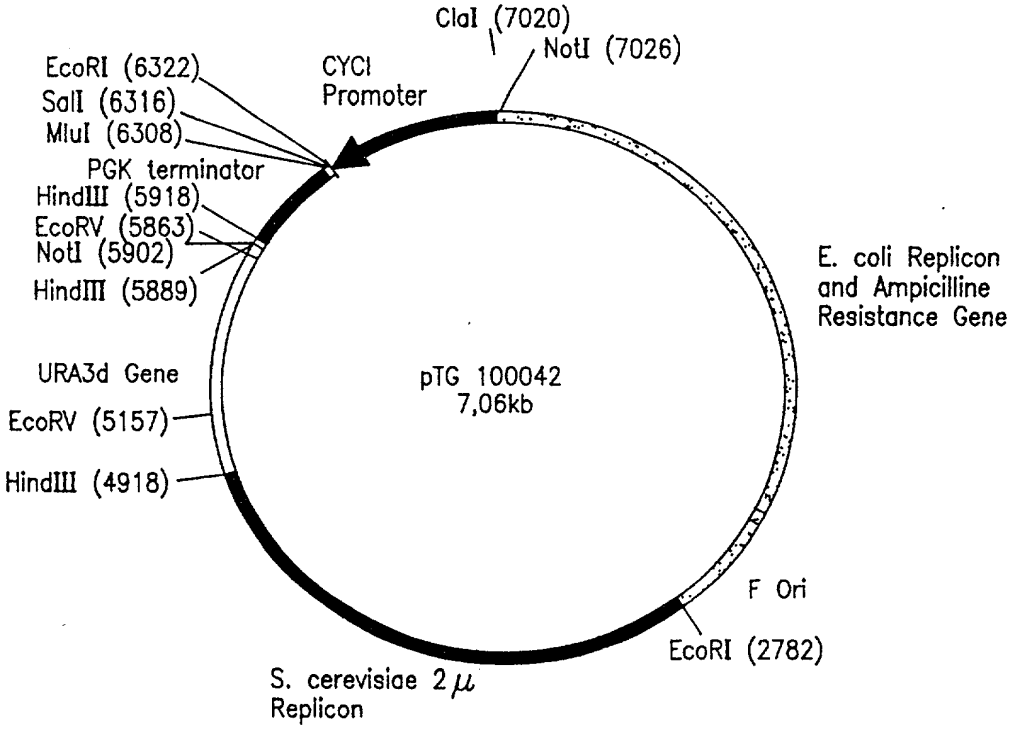


FIG. 52

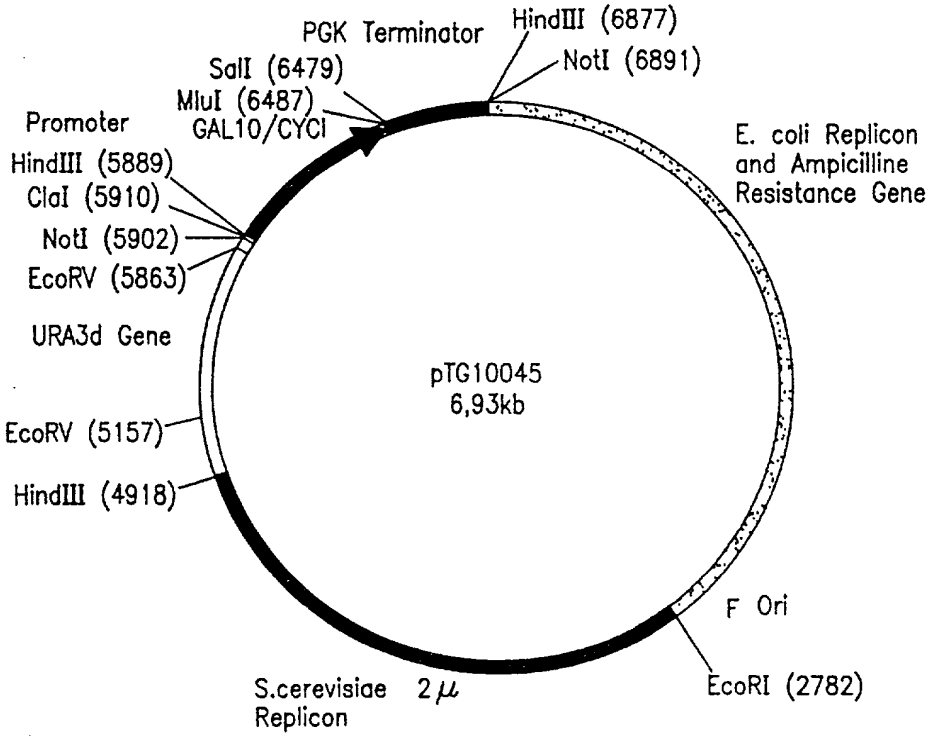


FIG. 53

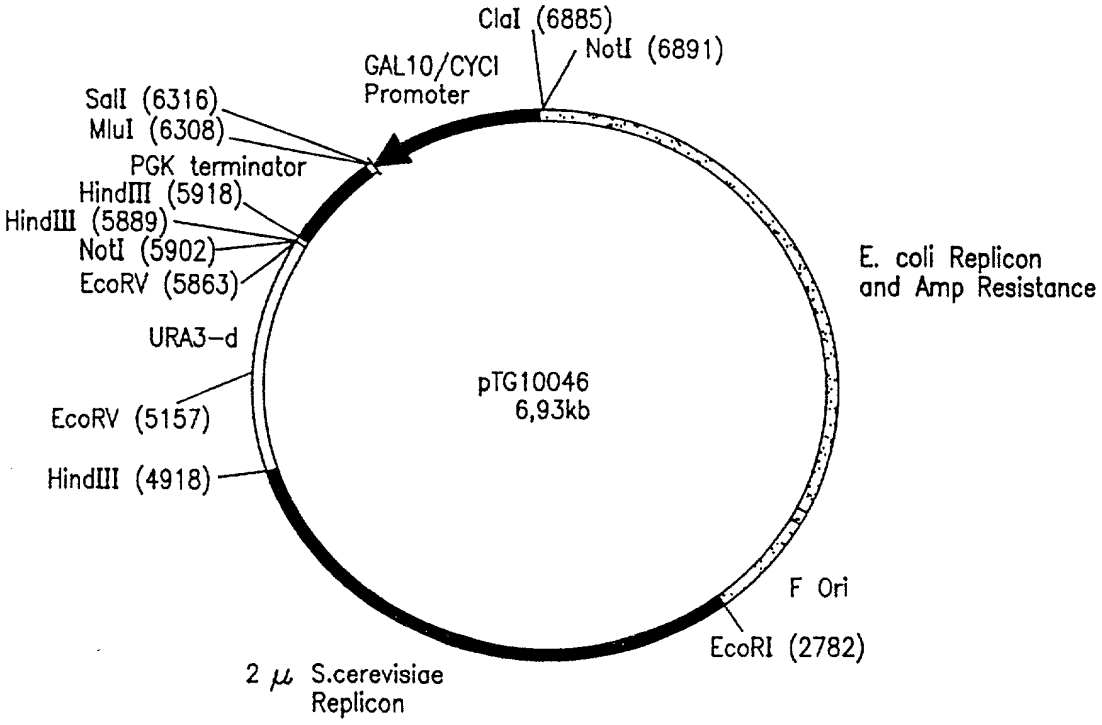


FIG. 54

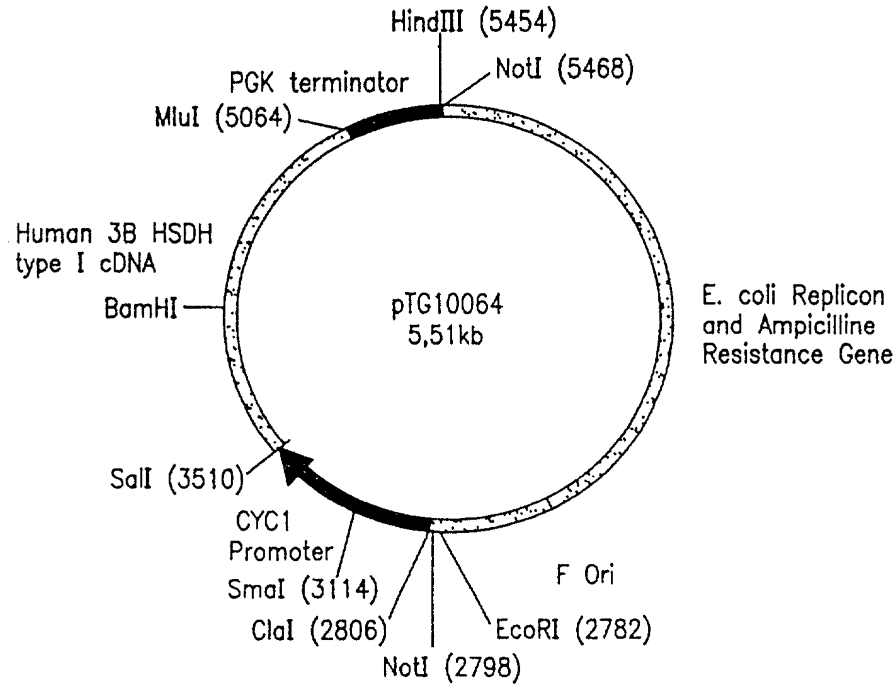

HindIII (5454)
NotI (5468)
PGK terminator
MluI (5064)
Human 3B HSDH
type I cDNA
BamHI
pTG10064
5,51kb
E. coli Replicon
and Ampicilline
Resistance Gene
SalI (3510)
CYC1
Promoter
SmaI (3114)
ClaI (2806)
F Ori
EcoRI (2782)
NotI (2798)

FIG. 55

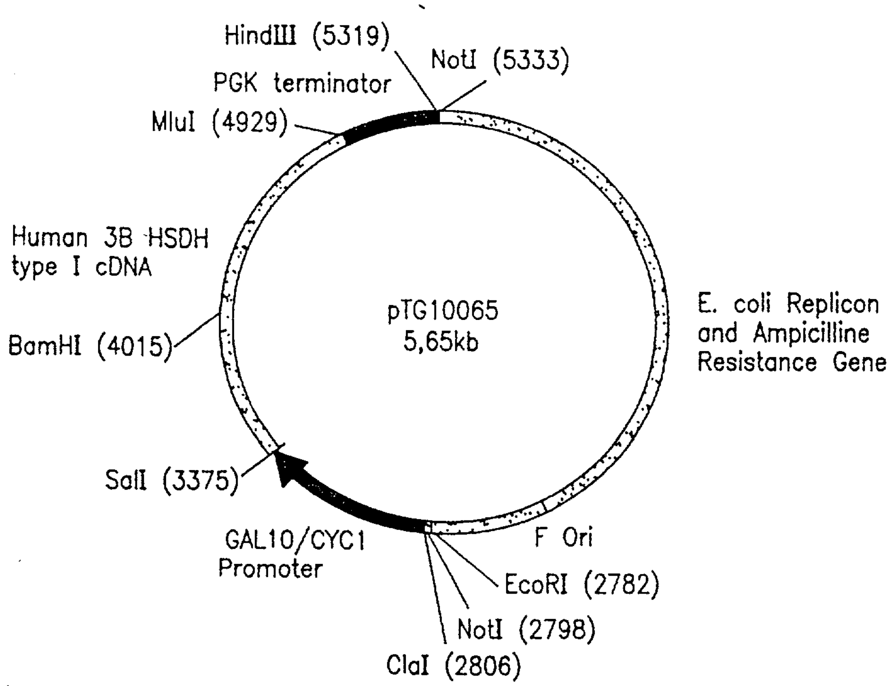

HindIII (5319)
NotI (5333)
PGK terminator
MluI (4929)
Human 3B HSDH
type I cDNA
BamHI (4015)
pTG10065
5,65kb
E. coli Replicon
and Ampicilline
Resistance Gene
SalI (3375)
F Ori
GAL10/CYC1
Promoter
EcoRI (2782)
NotI (2798)
ClaI (2806)

FIG. 56

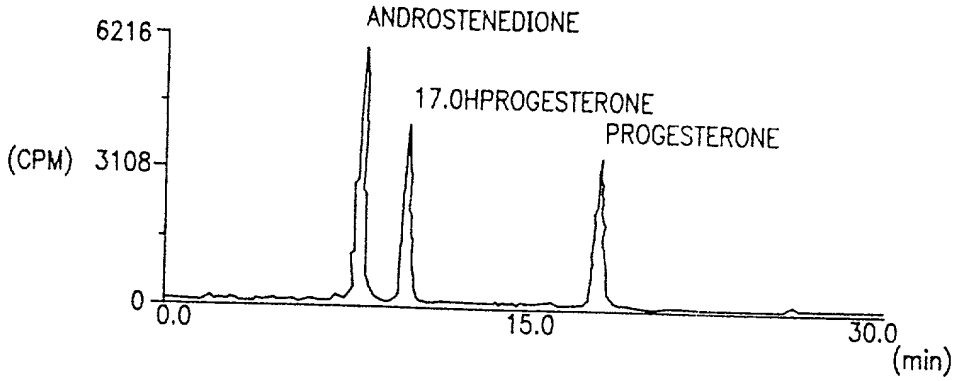


FIG. 57A

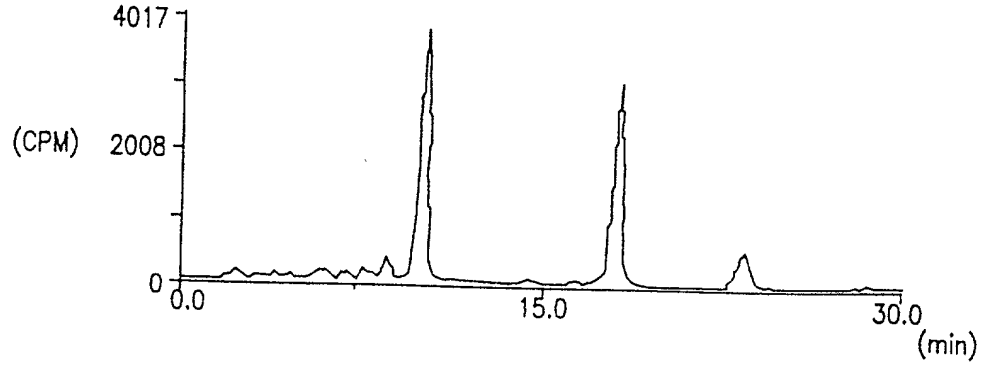


FIG. 57B